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Transparent exopolymer particles: detection and role in membrane based systems

Valerie Discart

Dissertation presented in partial fulfillment of the requirements for the degree of Doctor in Bioscience Engineering

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Examination committee:
Prof. dr. ir. J. Van Orshoven, chair
Prof. dr. ir. I. Vankelecom, supervisor
Prof. dr. ir. D. Springael
Prof. dr. K. Muylaert
Prof. dr. ir. I. Smets
Prof. dr. ir. N. Boon
(U Gent)

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[1] Coussement et al., 2010

Abstract

Transparent exopolymer particles (TEPs) have been a rather unknown component of the extracellular polymeric substances. Lately, they received more attention because of some remarkable characteristics, such as their transparency and their stickiness (defined as high probability of adhesion upon collision). Since the development of their detection method, they have been found to be ubiquitous in natural waters, where they are primarily formed from polysaccharides secreted by microalgae and bacteria. Several recent studies have pointed out an important role of TEPs in the biological and colloidal fouling of membranes during water purification. An important factor to be considered is that TEP particles are not formed in biofilms on surfaces, but in the bulk of the water itself. There they form microclimats for micro-organisms and can even form a kind of protobiofilm which can accelerate the fouling of surfaces significantly. The research performed on TEPs is hindered by the fact that there are several possible detection methods. Since the development of the first method to quantify TEPs in 1993, a number of alternative methods have been developed in an attempt to improve the original method. Some of these alternative methods deviate strongly from the original method and determine a different fraction of TEPs present in the same investigated sample. This makes comparison between results of studies very difficult or even impossible. In Chapter 1, an overview and a possible categorization of TEPs is presented to elucidate which fraction is measured by which method and how this fraction relates to the ones measured in other studies.

In this PhD, several systems where TEPs could be relevant were experimentally investigated. These sytems were (1) an ultrafiltration water purification plant, where TEPs were suspected to contribute to the irreversible fouling, (2) dead-end microalgae filtration for harvesting, where TEPs were hypothesized to be a determining factor in filtration, (3) a continuous microalgae growth and harvesting installation with submerged membrane filtration, where TEPs. along with other

factors, could have an influence on productivity and growth, and (4) a submerged membrane filtration system where parameters, such as membrane characteristics and coagulant addition, were varied for better microalgae harvesting.

A full-scale ultrafiltration installation, operated by The Watergroep at Harelbeke, Belgium, for the purification of surface water was monitored for 8 months with measurements of several parameters, including TEP quantity (Chapter 2). A correlation study and membrane autopsy showed a complex fouling mechanism with interactions between algae, iron and TEPs. Overall, algae related parameters, like chlorophyll concentrations, correlated stronger than TEPs with irreversible fouling rates. The presence of TEPs could hardly be detected on the membrane surfaces and their overall role in membrane fouling therefore seemed limited in the studied ultrafiltration plant.

Microalgae growth and harvesting is another area where TEPs could be of importance, since high correlations were found in natural environments between microalgae blooms and TEP-concentration peaks. Therefore, the role of TEP particles on membrane fouling during dead-end filtration of different *Chlorella vulgaris* broth solutions was investigated (Chapter 3). Different fractions of the broth (e.g. the soluble and bound fractions and the cells separated from these fractions) were also used as filtration feed. The relation between the feed properties and their filterability over three membranes was determined. The statistical analysis disclosed that no universal sample variable and fouling parameter could solely explain the filtration performance. However, soluble compounds, TEPs and carbohydrates, seemed of high importance for flux-decline when using low-pressure microfiltration membranes. This was inconclusive for ultrafiltration membranes, where the higher pressures presumably pushed all cells in a dense cake layer which determined the permeance.

In the area of microalgae growth and harvesting, membrane fouling is not the only important factor to be considered. TEP concentrations and other parameters were followed together with biomass density and productivity of microalgae in a membrane photobioreactor with continuous growth and harvesting of microalgae (Chapter 4). Results showed that TEPs were secreted during the algae cell growth, and that their presence is thus inevitable in a continuous system. In addition, substances such as counter ions and unassimilated nutrients accumulated in the system. Also bioflocculation was observed. Although the direct cause was not determined, it is likely that a combination of these factors limited the algae growth, which indicates that there is a limit on the number of time the growth medium can be recycled.

When microalgae harvesting is done with the aid of coagulants or flocculants, possibly in combination with membrane filtration, there is the possibility that negatively charged algogenic materials could interact with the positively charged coagulants. Supposing that membrane fouling is mainly due to algogenic material rather than algae cells themselves, filtration systems could be optimized using coagulants. In a last study, filtration tests were performed in an effort to determine optimal parameters for the preparation of membranes via phase inversion and for the addition of coagulants to obtain a maximal flux and minimal fouling during filtration of *Chlorella vulgaris* (Chapter 5).

In all, TEPs are a group of ubiquitous particles that, for multiple reasons, are not easy to define or quantify. Their role in the studied systems did not seem as large as was expected initially, based on earlier reports.

Samenvatting

Transparante exopolymeerpartikels (TEPs) zijn lange tijd een weinig bekende component van de extracellulaire polymere substanties geweest. De laatste jaren zijn ze echter steeds meer in de aandacht gekomen omwille van enkele markante eigenschappen, zoals hun transparantie, waardoor ze lange tijd aan detectie ontsnapten, en hun kleverigheid (hoge waarschijnlijkheid van adhesie bij botsing). Sinds hun detectiemethode ontwikkeld werd, zijn ze alomtegenwoordig gebleken in natuurlijke waters, waar ze voornamelijk gevormd worden uit polysachariden afgescheiden door microalgen en bacteriën. Verschillende recente onderzoeken wijzen op een belangrijke rol van TEPs in de biologische en colloïdale vervuiling van membranen bij waterzuivering. Van belang hierbij is dat ze niet worden gevormd in biofilms op oppervlakken, maar in de bulk van de oplossing zelf. Daar vormen ze microklimaten voor micro-organismen en kunnen ze zelfs een soort "protobiofilm" vormen die vervuiling van oppervlakken aanzienlijk kan versnellen.

Het onderzoek naar deze TEPs wordt echter bemoeilijkt doordat verschillende mogelijke detectiemethoden bestaan. Sinds de ontwikkeling van de eerste methode om TEPs te kwantificeren in 1993, zijn een aantal alternatieve methoden ontwikkeld in een poging de originele methode te verbeteren. Die methoden wijken soms sterk af van de originele methode. Dit maakt vergelijkingen tussen de resultaten van studies moeilijk of zelfs onmogelijk. In hoofdstuk 1 wordt een overzicht en een mogelijke categorizatie van TEPs gegeven om te verduidelijken welke fractie wordt gemeten door welke methode en hoe die fractie zich verhoudt tegenover deze gemeten in andere studies.

In dit doctoraat werden verschillende systemen onderzocht waar TEPs relevant konden zijn. Dit gebeurde voor (1) een ultrafiltratie waterzuiveringsinstallatie, waar onderzocht werd of TEPs bijdroegen tot de irreversibele vervuiling, (2) directe dead-end microalgenfiltratie, waar gekeken werd of TEPs een bepalende

factor konden zijn voor de realiseerbare filtratiesnelheid bij het oogsten van de algen via membraanfiltratie, (3) een continue microalgengroei en -oogstinstallatie met ondergedompelde membraanfiltratie, waar TEPs en andere factoren in het algenmengsel een invloed konden hebben op de productiviteit van de algen, en (4) een ondergedompeld membraanfiltratiesysteem voor microalgenoogst, waar niet meer gemonitord werd, maar actief parameters van membraanaanmaak en coagulanttoevoeging werden gevarieerd om een optimaal systeem te bekomen.

De industriële ultrafiltratie-installatie, uitgebaat door De Watergroep in Harelbeke, voor de zuivering van oppervlaktewater werd maandenlang gemonitord met metingen van verschillende parameters, waaronder TEPs (hoofdstuk 2). Een correlatiestudie en membraanautopsie toonden een complex vervuilingsmechanisme met interacties tussen algen, ijzer en TEPs. Over het algemeen correleerden algen-gerelateerde parameters sterker met de irreversibele vervuiling dan TEPs. Gedurende de membraanautopsie konden de TEPs amper waargenomen worden op de membraanoppervlakken en hun rol in membraanvervuiling leek dan ook beperkt voor deze ultrafiltratie-installatie.

Microalgenkweek en -oogst is een ander domein waar TEPs belangrijk kunnen blijken, gezien de sterke correlatie in de natuur tussen microalgenbloei en TEP-concentratiepieken. Daarom werd de invloed van de partikels op vervuiling van membranen tijdens dead-end microfiltratie van verschillende *Chlorella vulgaris* suspensies nagegaan (hoofdstuk 3). Verschillende fracties van de suspensie (o.a. de opgeloste en gebonden fracties en de cellen gescheiden van deze fracties) werden ook elk apart gebruikt als voedingsoplossing. De relatie tussen voedingseigenschappen en hun filtreerbaarheid over drie membranen werd bepaald. Uit een statistische analyse bleek dat er geen universele staalvariabele en vervuilingsparameter bestond die op zichzelf de filtratieperformatie kon verklaren of voorstellen. De opgeloste stoffen, TEPs en carbohydraten, leken echter wel van groot belang voor de fluxafname wanneer gebruik gemaakt werd van lagedrukmembranen. Dit was niet duidelijk het geval voor ultrafiltratiemembranen, waar de hogere werkingsdruk waarschijnlijk alle cellen in een dense cake laag duwde die de permeantie bepaalde.

In het domein van microalgenkweek en -oogst zijn echter nog andere zaken van belang dan alleen membraanvervuiling, zoals de groei van de microalgen zelf. TEP-concentraties en andere parameters werden gevolgd samen met de biomassadichtheid en productiviteit van microalgen in een membraan-fotobioreactor met continue groei en oogst van microalgen (hoofdstuk 4). De resultaten toonden dat de TEPs gesecreteerd werden de groeifase van de microalgen en dat hun aanwezigheid dus onvermijdbaar is in een continu systeem. Daarbij komt dat substanties zoals tegenionen en ongeassimileerde nutriënten accumuleerden in

het systeem. Ook bioflocculatie werd waargenomen. Hoewel de directe oorzaak niet werd vastgesteld, is het waarschijnlijk dat een combinatie van deze factoren de algengroei beperkte, wat inhoudt dat een limiet bestaat op het aantal keer dat het groeimedium gerecycleerd kan worden.

Wanneer bovendien de microalgenoogst gebeurt met behulp van coagulatie en flocculatie, eventueel in combinatie met membraanfiltratie, speelt nog een andere mogelijke invloed: de negatief geladen algogene materialen zouden met de positief geladen coagulanten of flocculanten kunnen interageren waardoor deze niet meer beschikbaar zijn voor coagulatie van de microalgen zelf. Op die manier, ervan uitgaand dat membraanvervuiling vooral toe te schrijven is aan algogeen materiaal in plaats van aan de algencellen zelf, kan een filtratiesysteem geoptimaliseerd worden met behulp van coagulanten. In een laatste studie werden optimale parameters onderzocht voor de aanmaak van membranen en toevoeging van coagulanten om een maximale flux en minimale vervuiling te bekomen bij de filtratie van microalgen (hoofdstuk 5).

Algemeen kan besloten worden dat TEPs een groep veelvoorkomende partikels zijn die om uiteenlopende redenen moeilijk te definiëren of kwantificeren zijn. Hun rol in de hier bestudeerde systemen was niet zo groot als verwacht werd gebaseerd op eerdere studies.

Abbreviations

AAS	Atomic absorption spectrometer
AB	Alcian Blue
AOM	Algogenic organic matter
ABSP	AB stained particles
APS	Acid polysaccharides
bTEP	Bound transparent exopolymer particles
C_{AB}	concentration of AB
CEB	Chemically enhanced backwash
$Cells^{EPS}$	Algae cells associated with their bound polymeric substances
Chl	Chlorophyll
Chla	Chlorophyll a
Chlb	Chlorophyll b
Chlc	Chlorophyll c
Chltot	Total Chlorophyll
CIP	Cleaning-In-Place
CSPs	Coomassie stained particles
CWP	Clean water permeance
cTEP	Colloidal transparent exopolymer particles
d	Day
D	Dilution rate
DLS	Dynamic laser scattering spectroscopy
DOC	Dissolved organic carbon
EPS	Extracellular polymeric substances
EPS_{CH}	Carbohydrate fraction of extracellular polymeric substances

EPS_{PR}	Protein fraction of extracellular polymeric substances
f_x	Calibration factor
HAMGM	Highly assimilable microalgae growth medium
IC	Inorganic carbon
IFM	Improved critical flux method
IFR	Irreversible fouling rate
IFR_L	Irreversible fouling rate calculated with permeance
IFR_R	Irreversible fouling rate calculated with resistance against filtration
J	Flux (L/m^2 h)
L	Permeance ($L/(m^2$ h bar))
L_{CW}	Clean water permeance ($L/(m^2$ h bar))
min	Minute
MMM	Mixed matrix membrane
MPBR	Membrane photobioreactor
n	Number of XG dilutions
OC	Organic carbon
PBR	Photobioreactor
$PC_{0.1}$	Polycarbonate filter with pore size of 0.1 μm
$PC_{0.4}$	Polycarbonate filter with pore size of 0.4 μm
PEK	Polyethylene membrane from Kubota
PES	Polyethersulphone
$PES_{5\ kDa}$	Polyethersulfone filter with molecular weight cut-off of 5 kDa
Pheo	Pheophytine
pTEP	Particulate transparent exopolymer particles
rpm	Revolutions per minute
scTEP	Soluble colloidal exopolymer particles

SEM	Scanning electron microscopy
SFCA	Surfactant-free cellulose acetate
SFV	Specific filtration volume
SMP	Soluble microbial products
SMP_{CH}	Carbohydrate fraction of soluble microbial products
SMP_{PR}	Protein fraction of soluble microbial products
spTEP	Soluble particulate transparent exopolymer particle
SS	Suspended solids
sTEP	Soluble transparent exopolymer particles
TC	Total carbon
TEPs	Transparent exopolymer particles
TEPtot	Total transparent exopolymer particles
TMP	Transmembrane pressure
TMP_F	Transmembrane pressure due to fouling
TMP_M	Transmembrane pressure due to membrane resistance
TOC	Total organic carbon
TOC_{feed}	Feed TOC
TOC_{filt}	Filtrate TOC
$Total_{CH}$	Total carbohydrates
$Total_{TEPs}$	Total TEPs
TTF	Time to filter
UF	Ultrafiltration
V_{st}	The volume filtered for staining (L)
V_f	Volume of filtrate
W	Dry weight of the standard (mg/L)
WC	Wright's Cryptophyte Medium
XG	Xanthan Gum

Contents

General introduction

It is common knowledge that water and energy are two of the most important resources for our human society. Water availability, as well as energy, impacts almost all aspects of the society, in particular health, food production and food security, domestic water supply and sanitation, industry and environmental sustainability. Yet, while global warming poses a threat to the easy accessibility of water and the world population increases, water use has been growing at more than twice the rate of the population increase in the last century [51]. For securing both water and energy supply, membrane technology can be a part of the solution. Membrane technology has evolved to become a predominant technology in diverse areas of industry, such as water purification, food and energy production. The predominant problem in most of these applications is membrane fouling. Membrane fouling can be caused by deposited particles or colloids, inorganic and organic components and micro-organisms (bio-fouling). Membrane fouling can reduce the membrane performance with more than 95% by causing a higher transmembrane pressure and thus a higher energy consumption, as well as a lower efficiency. Membrane fouling also shortens life span of the membrane because of the frequent physical and chemical cleaning processes that are required [39, 47].

One factor that can contribute significantly to membrane fouling are transparent exopolymer particles or TEPs. TEPs have long been known and are extensively documented in the context of oceanography [3, 50, 114, 115]. They are transparent gel-like particles that mainly consist of acid polysaccharides and are thus stainable by Alcian Blue (AB). TEPs are considered a class of extracellular polymeric substances (EPS) that, unlike normal EPS, appears in suspension and can be formed abiotically from free polysaccharide residues from several types of micro-organisms [114]. In 2005, Berman and Holenberg argued that TEPs in source waters can lead to biofilm growth on membrane surfaces. They elucidated the theoretical aspects of TEPs which make them highly probable as biofilm promotors: they are negatively charged polysaccharide particles that are very numerous in most waters, they are small and sticky, and many of

them already carry resident bacterial populations [19]. After that, a number of studies showed a possible link between TEP-occurence and membrane fouling in different set-ups, such as reverse osmosis [152], ultrafiltration (UF) membranes [79, 20] and membrane bioreactors [41]. Bar-Zeev et al. [15] suggested that the measurement of TEPs in different stadia of pretreatment could be an effective aid for the optimization of the treatment of wastewater and seawater. Before this to become a realistic option, there is need for more focused research to understand the characteristics of TEPs and their actual role in membrane fouling. Because TEP concentrations and characteristics vary seasonally and are dependent on the aquatic environment, it is important to know the characteristics of the TEPs in the different circumstances to develop the optimal pretratment for a certain application [15].

In the studies mentioned above, water purification is the main objective, but also in microalgae growth and harvesting, TEPs could have a significant impact. Microalgae are currently considered a promising source of biomass [33]. One of the reasons is that they do not require arable land, thus competition with food crops is not an issue. On top of that, many microalgae can be cultivated in low quality water, and some species can even grow in seawater or domestic waste water [128]. Furthermore, microalgae can produce more biomass per unit land area than agricultural crops, with in comparison to agricultural crops a higher proportion of useful products like fatty acids and proteins, and a lower amount of waste products like lignine or cellulose [104]. However, despite all the theoretical advantages of microalgae cultivation, there exist still some major challenges. One of the main problems is the large energy demand for harvesting the biomass, i.e. the separation of the microalgae from their growth medium, because of which the energetic cost of the process is not in proportion with the projected energy yield [98, 136, 156]. Traditionally, harvesting is performed through centrifugation, which is an efficient and proven method, but the high investment and operating costs render this method inapplicable for large-scale use [34]. Several other techniques have been under investigation, such as flocculation [141], electro-coagulation [143] and membrane techniques [26]. They have proven to be more energetically favorable solutions. Membrane-based harvesting processes could be further improved if the fouling of the membranes by the microalgae and their algogenic organic matter (AOM) is reduced [7, 83]. This AOM contains the main precursors of TEPs in natural environments [114]. A better understanding of the fouling of the membranes by TEPs could thus be a factor in the optimal design of membranes for the harvesting of microalgae.

As for the microalgae cultivation itself, there are also improvements that could be made. The main reason why harvesting of microalgae is such a major concern is that the maximum biomass concentrations during cultivation are generally low: from 0.14 g/L in open pond reactors to a maximum of 4 g/L

in photobioreactors [33]. If these concentrations could be kept as high as possible, without increasing the costs of cultivation excessively, the overall cost of cultivation and harvesting would be reduced. By coupling cultivation and harvesting in a membrane photobioreactor (MPBR) with permeate recycle, this could be achieved: improvements could be made with respect to productivity, efficient pre-harvesting of the biomass and a lower water footprint could be realized [26]. To optimize this MPBR system, the impact of TEP-production and several other parameters of the broth on productivity have to be evaluated.

Aim and outline

As mentioned above, TEPs are a relatively new factor in membrane fouling and microalgae research. As a consequence, there are a lot of questions that could be answered with regard to their role in water systems. Do they indeed cause membrane clogging even at very low concentrations? Do they promote biofilm formation in these circumstances? Are they more important than other algogenic material in determining microalgae filtration rate? In general, the aim of the present study was to determine the role of TEPs in a number of specific membrane-based processes, such as UF water purification and microalgae biomass production.

Because confusion currently exists concerning the definition of the term TEPs, as well as concerning the main quantification methods, a critical synthesis was necessary to clarify this. Therefore, Chapter 1 gives an overview of current literature on TEPs concerning the methods for their detection and quantification, and research on their link with membrane fouling.

Studies of TEPs in UF systems have been performed at lab-scale [20], or full-scale at short term [79]. Only one long-term full-scale study of the influence of TEPs in an UF plant has been performed, but only for sea-water as feed source [153]. To confirm the link between algae blooms and TEP peaks, and between TEP peaks and membrane fouling in a fresh water setting, a study on the long-term impact of TEPs on a full-scale fresh water UF plant was performed in Chapter 2.

As mentioned above, microalgae are a major source of the TEP precursors in natural environments, and at the same time the role of these specific particles in microalgae cultivation had never been investigated. One would expect that the particles would be present in significant amounts in microalgae cultivation broth and influence in an important way different steps of microalgae cultivation (the growth itself, biomass upconcentration by membrane clogging, ...). Therefore, in Chapters 3 and 4, two different settings were used to elucidate the possible

influences of TEPs. In Chapter 3, the last step in microalgae cultivation, namely the upconcentration using membrane filtration, was assessed for the possible fouling impact of TEPs during dead-end filtration of distinct microalgae broths, using *Chlorella vulgaris* as a model microalgae species.

In Chapter 4, TEPs and other components were monitored in an MPBR along with the growth and productivity of *Chlorella vulgaris*.

Finally, in Chapter 5, the focus returned to the upconcentration step itself. Costumizing a membrane for harvesting a particular microalgae species could be interesting due to the diversity of microalgae species with respect to cell size, cell wall chemistry and morphology. At the same time, a large number of membrane preparation parameters can be optimized. Using a submerged membrane filtration system, membranes were therefore optimized for microalgae harvesting, with and without coagulant addition (to quench membrane fouling AOM).

General conclusions are drawn in Chapter 6 and possibilities for future research are presented.

Chapter 1

Critical evaluation of the determination methods for TEPs, agents of membrane fouling

Adapted from: Discart, V., Bilad, M. R., and Vankelecom, I. F. J. Critical evaluation of the role of transparent exopolymer particles in membrane fouling and their determination methods. *Critical Reviews in Environmental Science and Technology*, accepted.

Abstract

Since the development of the first method to quantify transparent exopolymer particles in 1993, a number of alternative methods have been developed. Some of these alternative methods deviate firmly from the original method and in many cases, different methods determine a different fraction of the material present in the same investigated sample. This makes comparison between results of different studies very difficult or even impossible. Better categorizing of transparent exopolymer particles could be useful to keep a clear view on which fraction is measured and how this fraction relates to the ones measured in other studies.

1.1 Introduction

Transparent exopolymer particles (TEPs) are a class of organic particles that are ubiquitous in aquatic environments [114]. They can be considered as free-floating extracellular polymeric substances (EPS) that are composed mainly of acidic polysaccharides, with specific properties, such as a gel-like structure, transparency, stickiness and deformability [114, 162, 113, 96]. TEPs are actually microgels, part of a size continuum of organic matter in marine and freshwaters that ranges from polymers through nanogels to microgels to very large marine (or lake) snow particles [14]. TEPs have long been the subject of oceanographic and limnological (freshwater) research; especially since 1993 when the first TEP semi-quantitative determination method (Alldredge (1993) method) was proposed [3]. Before that, there were several reports about the occurrence microscopic particles in natural waters [82, 86], but they were never quantified. A few years later, a more practical and faster method was developed (the Passow and Alldredge (1995) method), which further led to an increase in TEP related research (Figure 1.1) [115].

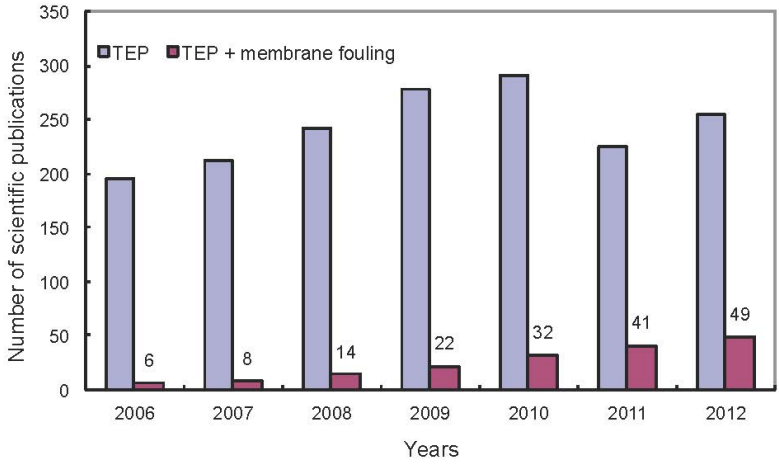

Figure 1.1: Trend in publications about TEPs and TEPs in membrane fouling. Until 2010, there was a constant increase of general TEP publications. The research about the involvement of TEPs in membrane fouling started around 2000 and increased until the year 2012 (search performed on the 1st of February, 2013).

The significance of TEPs in biogeochemical cycling of elements and the structuring of food webs has been the subject of investigation since they

were first detected [16, 13, 109]. TEPs adsorb trace metals and dissolved organic compounds. They are often loaded with bacteria and other microbes. This way, TEPs become 'hot spots' of intense microbial activity and chemical transformations within the water mass. Together with their associated flora and fauna of microorganisms, TEPs serve as 'food packages' for all kinds of small plankton and even for larval fish. Additionally, TEPs can aggregate with each other or with other small bits and pieces of detritus to form larger particles called marine or lake 'snow'. Some of the TEP and 'snow' sinks out of the upper water column and transports large amounts of organic matter and microorganisms to deeper water and sediments [114].

Later, their link with membrane fouling during filtration processes was suggested [19], which led to an increased focus of some membrane research groups on TEPs, especially to reveal their roles in diverse filtration systems. TEPs are suspected to induce colloidal fouling or biofilm formation, or both. The influence of TEPs was studied in a wide variety of membrane processes, including reverse osmosis [153, 152, 15], ultrafiltration (UF) [150, 20], and membrane bioreactors [41]. In all these systems, TEPs were found to play at least a partial role in membrane fouling. Apart from that, the importance of the particles can also be broadened to a lot of other aquatic biofilm-related problems, such as water distribution systems [14].

However, the accurate quantification of the particles still forms a major problem in TEP-related research. Some of the problems might be explained by the dynamic microgel properties of the TEPs, like their highly changeable size and shape in response to subtle physical or chemical changes [145]. After recognition of several limitations of the Passow and Alldredge (1995) method, several alternative methods were developed, mainly aiming at increasing reliability, reproducibility, and simplicity [152, 151, 135, 6, 35]. These methods are sometimes so diverse that the only feature in common is the use of Alcian Blue (AB) as staining agent. The use of diverse procedures not only has an impact on the comparability of the TEP quantities found in different studies (which is practically not feasible), but also on the notion of what TEPs actually are. Originally, TEPs had an operational definition, based on the Passow and Alldredge (1995) method, as particles that are retained on polycarbonate 0.4 μm filters, and stained with the cationic dye AB. The filter used, the pH and concentration of the AB solution were well defined [114]. Later methods do not always involve filtering of the solution and thus do not limit TEPs to the category of particles larger than the pore size of the polycarbonate 0.4 μm filters. Also the pH and AB concentrations of the staining solution have been varied. In this study, a critical overview is given of the different methods that exist in literature and the different definitions of TEPs they imply. This review is supplemented by some of our own experimental data. In addition, an attempt

was made to group the different established methods to have a more systematic overview.

1.2 Origins of TEPs

Before discussing the actual methods, attention will be given to the diverse origins of the TEPs, since this is important to understand the difficulties in characterizing and quantifying these particles. TEPs have been described as a class of particulate organic, acidic polysaccharides, which can be stained with AB. They have sticky and gel-like characteristics, are deformable and appear in various forms, e.g., amorphous blobs, strings, films, sheets, clouds, or clumps [15], and sizes (as a part of the size continuum of organic matter in aquatic environments [14]). They have been found to be ubiquitous in surface water, seawater, and wastewater [113, 139].

In natural environments (and in membrane systems), TEPs and TEP precursors can be formed via either biotic or abiotic processes (Figure 1.2). In the biotic process, TEPs are generated directly by some kind of phytoplankton and bacteria: the TEPs then form from bacterial mucus or from multicellular organisms, like macroalgae, oysters or sea snails, or from human debris [65, 99]. However, in natural environments, microalgae, and especially diatoms, produce the majority of the TEP precursors, which form TEPs through abiotic processes [16, 50, 113]. These precursors, polysaccharide fibrils of 1–3 nm diameter and hundreds of nanometers long which can pass through 8 kDa pore size membranes, are secreted by the micro-organisms or produced through lysis or breakage of cells [86]. Processes such as coagulation, gelation and annealing of the dissolved precursors, give rise to submicron gels, which can in turn break down or assemble via coagulation or agglomeration to form colloidal TEPs, and in a further step to particulate TEPs (cTEPs and pTEPs respectively, see 1.4.1) [112]. This process is stimulated under specific environmental conditions, e.g., turbulence, ion density, and concentrations of inorganic colloids [114].

After staining with AB, the amount of TEPs is directly proportional to the amount of stain binding to it [121]. AB is a hydrophilic cationic dye that stains both carboxylated and sulfated polysaccharides at pH 2.5 [115]. In fact, AB can form complexes with anionic carboxyl (COO) and half ester sulfate (OSO_3) groups of all acid polysaccharides. However, several conditions can have an impact on the properties of the microgels-AB interactions, as well as on the structure and size of the TEP particles themselves. Temperature, pH and pressure can drastically affect the dissolved organic carbon self-assembly [145]. TEPs of different origins can also differ in size, composition, hydrophobicity,

structure and stability of the gel particles [22, 145]. This variability in properties in response to different conditions is of great importance, since measurements at different times with slight variations in e.g. temperature, could thus yield different results, depending on the origin of the TEPs present. As will be pointed out later, AB may also react differently under different conditions of e.g. salinity, pH or concentration.

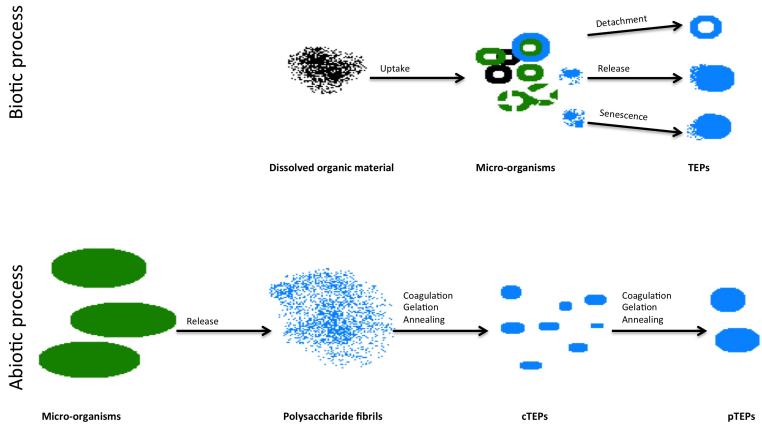

Figure 1.2: Overview of the different origins of TEPs.

1.3 AB and the rationale behind its TEP staining

AB is a bulky dye (see Figure 1.3), not very easy to work with, but it has been widely used in medical and biological research, e.g. to stain mucous layers, glycosamines in blood or urine, etc. [111, 121, 155]. Applicability in marine systems, however, had been limited because artifacts are formed in the presence of salts. Although the use of AB for selective staining started back in 1950, its chemical structure and staining mechanism was only revealed in 1972 [127]. AB consists of copper-phtalocyanin with four methylene-tetramethyl-isothiouronium-chloride side chains. Its ionic groups are effectively isolated from the rest of the molecule, thus their charges concentrate in a very small volume,

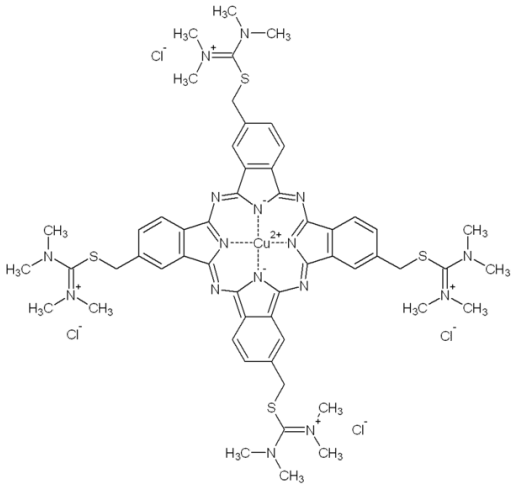

Figure 1.3: Molecular structure of AB. The dye consists of a copper-phtalocyanin core structure with four methylene-tetramethyl-isothiouronium-chloride side chains. Its ionic groups are effectively isolated from the rest of the molecule, thus reducing steric hindrance during the reaction with acid polysaccharides and forming a stable salt that sustains rather harsh post treatment.

creating a relatively high charge density, hence high reactivity. That makes them hard cations according to the Hard Soft Acid Base Theory . Carboxylic and sulfonic acids are hard anions. When a hard cation (AB) meets a hard anion (mucopolysaccharides), a very stable salt forms that cannot be de-stained by subsequent procedures (washing, additional staining with other dyes). This explains why AB is highly selective for tissue substances (given the proper solution pH), and forms insoluble complexes that withstand harsh subsequent treatment (like Periodic Acid Schiff-staining) without de-staining. Alternative basic dyes are attracted to negative groups in tissue, but very poorly match in geometry. Therefore, they do not bind sufficiently tight to withstand subsequent staining procedures or dehydration. AB is most often used to selectively stain acidic mucosubstances. At pH 1.0, AB stains only sulphated glycosaminoglycans and glycoproteins. At pH 2.5, it also stains hyaluronic acid and glycoproteins that owe their acidity to sialic acids. Glycosaminoglycans are colored more intensely at pH 2.5 than at pH 1.0 because their ionized uronic acid groups add to the negative charges of the sulfate half-esters. In other applications, AB is also used at pH 5.5, but its staining is restrained by adding different concentrations of an inorganic salt, e.g. $MgCl_2$ [80].

1.3.1 Alternative dyes

Many recent methods were developed to limit the vulnerability of dealing with AB. In addition, the harmful production process and limited availability of AB in certain periods of the last century had motivated researchers to look for alternatives [38]. However, a dye with the same selectivity (high for acidic polysaccharides, low for nucleic acids and proteins) and working properties has unfortunately not readily been found yet. Two other dyes out of thousands listed in the Colour Index and Conn's Biological Stains are similar to AB, namely Alcian yellow (the only one familiar to histologists having yellow colour) and basic red 18 (having a deep orange color). Unfortunately, they are no practical substitutes for AB because of their limited availability. Furthermore, the ability of the insoluble complexes of those dyes to withstand the subsequent treatments that are common in the current TEP procedure has still not been tested.

1.4 TEP determination methods

1.4.1 TEPs: "Particles retained by polycarbonate filters and stainable with AB"

This definition mainly includes the TEPs measured using the Alldredge (1993) method [3] and the Passow and Alldredge (1995) method [115]. Villacorte et al.[151, 152] proposed some changes, especially concerning the pore size of the polycarbonate filters and the calibration method (usually performed with standard solutions of Gum Xanthan (XG). In Table 1.1, some detailed characteristics of these methods can be found.

The Alldredge (1993) method

Before Alldredge et al. [3] published their method, researchers had been aware of the presence of transparent particles. TEPs had been observed and identified qualitatively from filtered and stained seawater by using the Periodic Acid Schiff method [57], but no quantification had been performed. Alldredge et al. [3] quantified them and revealed that they consist of acid polysaccharides. The quantification was performed by filtering a liquid sample through a 0.4 μm polycarbonate filter. The TEPs were afterwards made visible by staining the filter for $<$2 s with an aqueous solution of 0.06% acetic acid and 0.02% AB. The particles were then transferred to a slide and the filter was removed using the Filter-Transfer-Freeze technique (a method developed in 1983 to prepare

Table 1.1: Overview of TEP concentrations in literature and this study (expressed in particles/mL (number of particles per mL), XG/L (Xanthan Gum equivalents/L) or absorbance units/L (abs/cm/L).

Method	Sample type	Concentration	Reference
Alldredge 1993 method	Seawater (Santa Barbara Channel)	28 to 403 particles/mL	[3]
	Seawater (Monterey Bay)	120 to 4925 particles/mL	[3]
	Diatom culture *Chaetoceros gracilis*	4215 to 16,883 particles/mL	[3]
	Diatom culture *Nitzschia angularis*	814 to 1122 particles/mL	[3]
Passow and Alldredge 1995 method	Seawater (Santa Barbara Channel)	14-252 μg XG/L	[115]
	Seawater (Monterey Bay)	3-310 g XG/L	[115]
	Diatom culture *Chaetoceros gracilis*	1000 μg XG/L	[115]
	Diatom culture *Thalassiosira rotula*	706 μg XG/L	[115]
	Diatom culture *Emiliania huxleyi*	920 μg XG/L	[115]
	Seawater (Mediterranean Sea)	230-478 μg XG/L	[12]
	Seawater (Mediterranean Sea)	760-542 μg XG/L	[16]
Villacorte method	Secondary wastewater effluent	1572 μg XG/L*	[139]
	Surface water	698.8 μg XG/L*	[139]
	Ground water	below detection limit (50 μg XG/L)*	[139]
	Coastal seawater (Wadden Sea, Netherlands)	1.62 mg XG/L#	[152]
	River water (Meuse, Netherlands)	270 μg XG/L#	[152]
	Blend of brakish and sea water (Oosterschelde estuary and the North Sea)	20-165 abs/cm/L**	[153]
	Seawater (Scheveningen coast)	8.1 mg XG/L	[151]
	Reservoir water (De Biesbosch)	0.7 mg XG/L	[151]
	Marine diatom culture - *Chaetoceros affinis*	20-180 abs/cm/L	[151]
	Freshwater Blue-green algae culture - *Microcysitis* Sp.	8-35 abs/cm/L	[148]
	Freshwater green algae culture - *Chlorella vulgaris*	2.3-33 abs/cm/L	[46]
Arruda Fatibello method	Freshwater (Barra Bonita reservoir)	240-400 μg XG/L	[6]
Thornton method	Seawater (Gulf of Mexico)	0.1-4.5 mg XG/L	[135]
	Freshwater (ponds on campus of Texas A&M University)	2-22 mg XG/L	[135]

*calibration factor determined with TOC measurements
#calibration factor adopted from literature
**no calibration factor used

filtered samples for light microscopy [68]) . The sizes and abundances of the phytoplankton and TEPs (D > 3 μm) on each slide were determined using standard light microscopy at 200x magnification. In this first TEP quantification, TEPs were found to range from 28 to 5000 particles/mL and to vary from 5 to 50 μm in length [3]. The Filter-Transfer-Freeze technique is quite laborious, slow and complex. Logan et al. [89] introduced a much easier and faster method using clearing slides (cyto-clear slides, also used by Berman and Parparova [21], see below), by which phytoplankton can be viewed directly on the polycarbonate filters under brightfield illumination.

The Passow and Alldredge (1995) method

The microscopic determination of TEPs using the Alldredge (1993) method is labor-intensive and slow, especially since the stained particles often did not have high enough contrast to use image analysis techniques in 1995 [115]. Image analysis has later been successfully used to quantify TEPs with the microscope method [22, 95]. However, Passow and Alldredge (1995) proposed a still faster and easier semi-quantitative method to determine the amount of TEPs present in seawater or freshwater, based on the colorimetric determination of the AB-TEP complexes. The first steps of the quantification are the same as in the Alldredge (1993) method, i.e. the filtering of aqueous samples through 0.4 μm polycarbonate filters with 24 mm diameter, after which the TEPs are stained for less than 2 s with an aqueous solution of 0.06% acetic acid and 0.02% AB. After the staining, the filters are rinsed once with distilled water to remove excess dye. Filters are then transferred into a 25-mL beaker containing 6 mL of 80% H_2SO_4, soaked and intermittently shaken for 2 h, to release the AB into the solution. The absorption of the resulting solution is then measured using a spectrophotometer at a wavelength of 787 nm. TEP concentrations were calibrated against known concentrations of a "model" polysaccharide, xanthan gum (XG). The calibration was performed because AB is known to show marked batch variation in both purity and solubility. The calibration standard was prepared by making a uniform XG solution of 100 mg/L. Different amounts of solution were then filtered onto pre-weighed filters and then stained by the aforementioned procedure. The calibration factor was obtained by relating dry weight of XG particles to their staining capacity, as shown in Eq. 1.1.

$$f = W[(E_{st_{787}} - C_{787})V_{st}^{-1}]^{-1} \tag{1.1}$$

in wich W is the dry weight of the standard (μg/L), $E_{st_{787}}$ its average absorption, C_{787} the absorption of the blank, and V_{st} the volume filtered for staining (L). In their study, Passow and Alldredge found TEP concentrations in seawater

ranging from 10 to 500 μg XG/L depending on the season, depth and plankton community composition. In addition, they also tested the sensitivity of the method to several possible variations, such as: a) the AB concentration of the staining solution: concentrations lower than the 0.02% resulted in understaining, but higher concentrations did not lead to overstaining; b) the effect of the pH of the dye solution on the staining capability of AB: at a pH lower than 2.5, the staining was lower because polysaccharides with carboxyl groups do not stain (only sulfonated polysaccharides stain fully); c) staining time: a longer staining time did not result in higher absorption, unless phytoplankton cells were used, which lyse after 5 seconds at such low pH, resulting in stainable products; d) soaking time in H_2SO_4: small bubbles formed during decomposition of the organic materials impeded the absorption reading for the first 2h, then remaining stable for more than 20h; e) rinsing: no measurable difference was observed between rinsed and unrinsed samples; f) volume of staining solution: a larger quantity of staining solution did not lead to overstaining; g) filtration pressure: modifications in this parameter produced the greatest variation in the measured amount of particles; h) formalin-preservation: no interference with the staining procedure itself was found, but it can lead to an overestimation of the amount of TEPs when working with organisms that lyse in the presence of formalin; and i) freezing: no differences in the amount of TEPs measured.

The Passow and Alldredge (1995) method was a significantly improvement compared to the previous Alldredge (1993) method and provided a breakthrough for TEP quantification. However, there are still a few limitations associated with it: a) the procedure is quite laborious and requires patience and skill to avoid experimental errors, especially when analyzing water containing very low or very high TEP concentrations. The high chance for experimental error with high TEP concentrations has been partly addressed by the limitation of the result to absorbances lower than 0.4 (to avoid clogging of the filter); b) the staining of intracellular components, as demonstrated by Thornton et al. [135]; c) a major problem is that the TEP concentration is calibrated against a "standard" polysaccharide, XG. Therefore, it is impossible to express the TEP concentrations in any absolute form (e.g. as μgC/L). Attempts have been made to determine transformation factors [50], but these remain problematic; and d) the calibration is very difficult since the weight of the XG that has to be used for calibration is extremely low. Villacorte et al. have addressed some of the limitations in 3 sequential steps [151, 152, 153] (see below). It should be emphasized that only the Passow and Alldredge (1995) method has been very extensively used in quantifying TEP concentration (in more than 100 publications, mainly in oceanography, see Figure 1.1) and that this method is the only current method capable of detecting low TEP concentrations (<100 μg XG/L), such as frequently occurring in seawater. Only a very limited number of studies have used the Arruda Fatibello or Thornton methods (see below).

The Villacorte (2009a) method

Villacorte et al. [151] first pointed out the limitations of the Passow and Alldredge (1995) method, starting with the fact that a large fraction of the TEPs can be smaller than 0.4 μm. Passow and Alldredge recognized that there was a large fraction of <0.4 μm AB staining material in seawater, to which they referred as 'dissolved TEP precursors' (Passow, 2000). In limnological research, material that passes through 0.2-0.4 μm filters is commonly operationally defined as "soluble". Villacorte et al. [151] proposed to categorize TEPs based on their size in line with the IUPAC definition (where particles with a size range from 0.001 to 1 μm are considered colloidal): the TEPs obtained by the Passow and Alldredge (1995) method were called 'particulate TEPs' (pTEPs) and the TEP fraction passing through a 0.4 μm PC filter, but retained by 0.05 μm PC filter 'colloidal TEPs' (cTEPs). The 0.05 μm filter was chosen as the smallest possible filter, because pre-filtering of the AB solution with smaller filters reject 96% of the AB concentration [151]. Villacorte et al. [151] indeed found that 65-92% of the TEPs in surface water are cTEPs. Another change was the use by Villacorte et al. [152] of 47 mm diameter filters rather than the 24 mm diameter filters of Passow and Alldredge (1995) (and in most oceanographic TEP studies). Also, sample staining was done with 1 mL staining solution instead of 0.5 mL.

In the Villacorte (2009a) method, the calibration of the staining solution was done differently. A standard solution was prepared by mixing 20 mg of XG in 200 mL of ultra-pure water and then homogenised using a tissue grinder. Consequently, 40 mL volumes of 4–5 dilutions of the standard solution were prepared. For each dilution, 20 mL was filtered through 0.2 μm polycarbonate filters using an adjustable vacuum pump set at 0.2 bar of vacuum. The filtrate and remaining 20 mL of the feed were set aside for total organic carbon (TOC) measurements. The retained XG on the polycarbonate filter was stained with 1 mL of pre-filtered (0.05 μm polycarbonate filter) AB staining solution and further handled, as described by Passow and Alldredge (1995) (see higher). The calibration factor (f_x) was computed by relating the weight of XG to the absorbance of the eluted stain in the acid solution following equation 1.1, apart from the fact that the calibration factor is divided by the volume filtered for staining (V_{st}). The average retained dry weight of XG was estimated by computing the retained TOC as the difference between the feed TOC (TOC_{feed}) and the filtrate TOC (TOC_{filt}) per unit volume of filtrate (V_f) and then converted to equivalent weight of XG based on its molecular composition ($C_{35}H_{49}O_{29}$):

$$W = \frac{V_f}{0.45 n_{max}} \sum_{n=1}^{n=n_{max}} TOC_{feed} - TOC_{filt} \tag{1.2}$$

in which W = dry weight of the standard (mg/L), and n = number of XG dilutions. This calibration method was adopted by Kennedy et al. [79].

The Villacorte (2009b) method

In a later study, Villacorte et al. [152] monitored AB concentrations (C_{AB}) of the dye solution using an atomic absorption spectrometer (AAS) to measure the copper concentration (C_{Cu}), from which the C_{AB} could be calculated as:

$$C_{AB} = \frac{C_{Cu}}{0.0489} \tag{1.3}$$

Also, some problems with the calibration procedure were pointed out: (1) weighing of XG is very sensitive to inaccuracies because of the very small quantities on the polycarbonate filters and (2) preparation of XG solutions with uniform properties is very difficult. Therefore, a different approach was used to verify whether the AB solution had sufficient staining capacity. Reduction of staining capacity was observed when the AB concentration was below 150 mg AB/L (final concentration after pre-filtering: 75 mg/L). However above 200 mg AB/L, there was always enough staining capacity to stain all TEPs (final concentration after pre-filtering: 100 mg/L). Understaining of TEPs is thus unlikely. Comparison of the results from this study with other studies was sometimes not possible because of the use of different analytical methods to quantify the AB (the Passow and Alldredge (1995) method uses calibration by XG weighing, but the Villacorte method applies AAS). Otherwise, pTEP concentrations seemed to be generally comparable to TEP concentrations found in former studies [114, 151]. In this study, cTEPs were found to contribute to around 60-90% of the total TEPs [151].

The Villacorte (2010) method

Since the staining capacity remained constant above a certain AB concentration, the calibration using XG was deemed no longer necessary. Therefore, Villacorte et al. decided to drop the calibration method altogether [153]. Furthermore, TEP concentrations were no longer expressed in XG equivalents, but in terms of absorbance per cm of eluted AB in sulfuric acid per liter of filtered sample volume (abs/cm/L). When analyzing saline samples, an additional rinse with 1 mL of ultrapure water through the TEP gels prior to staining is necessary in order to replace the residual saline water adsorbed to it [148]. In some other studies the Villacorte method was adopted [139, 46]. Van Nevel et al. [139] used the calibration method with TOC measurements from the Villacorte (2009a)

method, while mentioning that the results obtained this way are relative and the quantifications expressed as XG equivalents were only used as visualization of the results. Since the amount of AB as determined by AAS was highly correlated (R^2=99%) to the absorbance of the solution at 787 nm, Discart et al [46] used the absorbance as an easy way to determine the AB concentration of the dyeing solution. Discart et al. [46] did not use a calibration method and expressed the TEP concentrations in abs/cm/L.

1.4.2 TEPs: 'Particles and soluble matter stainable with AB'

The methods described above all have in common that the particles are filtered before they are stained. Arruda Fatibello et al. and Thornton et al. used a different approach by staining the particles in the solution after which the AB-TEP complexes are separated and quantified spectrophotometrically. A practical definition for TEPs when applying the methods of Arruda Fatibello et al. and Thornton et al. [6, 135] is: all particles AND soluble matter stainable with AB. This definition has not been postulated anywhere yet, but is implied by the way the particles are determined. These methods do not filter before staining. By this definition, also the acidic polysaccharides (APS), determined by Thornton et al. [135], can be classified as TEPs, which is why this method is also mentioned here. It is important to note that these methods have been used only few times in comparison with the Passow and Alldredge (1995) method, presumably because both methods are much less sensitive compared to the widely used Passow and Alldredge method. Table 1.2 shows some characteristics of these methods.

The Arruda Fatibello method

The Arruda Fatibello method was presented as being simple and rapid, reliable, precise, and accurate and without the need for extensive sample pretreatment [6]. This method was originally used to quantify TEPs in fresh water samples, and is not applicable for sea water samples (which initially was the most studied TEP source), as salts can form insoluble complexes with AB [6]. However, with an additional procedure for removing salts, the method could in principle also be applied for sea water samples. The Arruda Fatibello method is based on the fact that the TEPs in solution react with excess AB yielding a hardly soluble AB-TEP complex, which settles down after centrifugation. The resulting absorbance of the excess AB is therefore inversely proportional to the TEP concentration. Typically, 2 mL of a freshwater sample is stained with 0.5 mL of a 0.06% AB solution after addition of a 0.2 mol/L acetate buffer solution until a final volume of 10 mL (pH 4). Afterward, the mixture is stirred for 1

Table 1.2: Characteristics of the TEP determination methods using a polycarbonate filter and AB.

	Alldredge method	Passow & Alldredge method	Villacorte method
Analysis characteristics			
Detection limit	1 particle/filtered amount **	around 2 μg XG eq/L **	around 50 μg Xeq /L **
Precision	around 50% (800 particles/mL)	around 5%	around 5%
Sample pretreatment	No pretreatment necessary, fixation with formalin possible ***	No pretreatment necessary, fixation with formalin possible ***	No pretreatment necessary, fixation with formalin possible ***
AB solution	0.02% AB, 0.06% acetic acid	0.02% AB, 0.06% acetic acid	0.02% AB, 0.06% acetic acid
Filter used	Polycarbonate 0.4 μm	Polycarbonate 0.4 μm	Polycarbonate 0.4 μm to 0.05 μm
Interferences			
Salts	None	None	None
Humic substances	None	None	None
Amino acids	None	None	None
Intracellular compounds	No/Yes*	No/Yes*	No/Yes*
Method characteristics			
Simplicity	Labor intensive	Medium	Medium
Speed	Slow	Medium-Slow (depending on the sample)	Medium-Slow (depending on the sample)
Optimization	Medium	Medium	Medium
Implementation	Medium	Medium	Medium
Cost	Medium	Medium	Medium

* According to Passow and Alldredge [115], no intracellular compounds are stained if the procedure is performed well; according to Thornton et al. [135], intracellular compounds are also measured by these methods.

** Depends on the amount filtered and the presence of disturbing non-transparent particles present [109]

*** Care must be taken with organisms that disintegrate in presence of formaline .

min and centrifuged at 3000 rpm (2160 g) for 30 min. The absorbance of the supernatant (excess AB solution) is measured at 602 nm (maximum absorbance of AB in water, as opposed to 787 nm for AB in sulfuric acid) to determine the amount of AB that has formed the complex with TEPs. The authors found that the optimum conditions, which gave the lowest detection limit and the greatest linearity (0.50-10 $\times$ 10^3 μg XG/L), were an AB concentration of 3 $\times$ 10^{-3}% (m/v), a pH of 4 and a stirring time of 1 min. Repeatability of the test was very good [6]. The pH change to 4 is quite remarkable since staining with AB is usually done at pH 1 or 2.5, depending on the material targeted [50, 80, 116, 157]. It is unclear which components AB stains at pH 4. It could be that this is not confined to acidic polysaccharides, but also includes carboxyl groups associated with proteins and, at elevated temperatures or after prolonged staining, also nucleic acids (Hayat, 2000). Interestingly, Arruda Fatibello et al. [6] selected pH 4 on the basis of linearity of the calibration curve rather than considering the materials stained under those conditions. They used XG, which is stained both at pH 2.5 and pH 4. Therefore, when adopting the Arruda Fatibello method and to be comparable with other methods, we strongly suggest to use pH 2.5.

The method of Thornton et al. (2007)

Thornton et al. [135] presented a simple method using AB to stain and quantify acid polysaccharides (APS) in water samples. The staining conditions are similar to the earlier established methods (Alldredge (1993) and Passow and Alldredge (1995) methods), but with some important differences. APS are first stained and precipitated with 1 mL of a 0.02% AB solution with a pH of 2.5, after which the precipitate is retained on a 0.2 μm pore size filter and the absorbance of the filtrate is measured at 610 nm using a spectrophotometer. Here, like in the Arruda Fatibello protocol, the color of the filtrate is inversely proportional to the amount of APS in the sample. The calibration can be done with XG or alginic acid as a standard. Thornton et al. [135] followed the previous definition of TEPs as being particles $>$ 0.4 μm, and decided to place the fraction they measured under the broader nominator 'acidic polysaccharides'. However, the measured fraction would probably be about the same in size as measured in the Arruda Fatibello method, where they call it 'TEPs', but not in composition because of the difference in operational pH. The authors claimed that this method is useful for studies on the biogeochemistry of particulate and dissolved APS, including TEPs and their precursors [135], and one could imagine its application to membrane fouling research as well.

The interference of salts with the assay is addressed by dialyzing the samples from marine environments before staining with AB. For the desalting of seawater

samples, different dialysis tubings were tested (namely with molecular weight cut-off of 12,000-14,000, 1000 or 100 Da) to ensure minimum loss of APS. The 1000 Da tubing was found most effective. A smaller molecular weight cut-off was unreliable as the tubing often took up water by osmosis and made the dialysis bags turgid.

Several other parameters were checked for this method. First, the absorbance signal was linear up to 0.0067% AB. Furthermore, the AB to sample ratio was tested, because it could affect the range of the assay (reducing the AB concentration at relatively low APS concentrations may increase accuracy and precision). The dye binding time (the time that the sample was exposed to AB before it was filtered) did not affect the results of the assay, suggesting that the APS-AB complex precipitated instantaneously. Sample storage with formalin also did not affect the calibration measurements (as in the Passow and Alldredge method (1995)). The pore size and material of the used filter were varied. It was found that there was no difference in amount of material retained by 0.1 and 0.2 μm polycarbonate filters. This result suggests that even small APS molecules would be retained since the APS-AB complex is not stable and precipitates easily. Therefore, the APS obtained via the Thornton method includes the cTEPs and the dissolved TEPs. As for the filter material, surfactant-free cellulose acetate (SFCA) syringe filters were tried instead of polycarbonate, because the polycarbonate filters were not so convenient to use as enclosed syringe mounted filters. SFCA filters were found to retain significant amounts of AB in the absence of APS, particularly at high stain concentrations. To overcome this problem, the first few mL of filtrate that come through an SFCA filter should not be collected and only the last 1 mL of the filtrate should be collected in the cuvette for absorbance measurement.

XG (Figure 1.4 A) was initially chosen as the standard for the assay as this has become the standard of choice for calibrating TEP assays. However, alginic acid (Figure 1.4 B), with very similar properties, can also be used for calibration [115, 135]. In fact, Hung et al. [73] suggested that alginic acid could be a more appropriate standard for APS, as it is less sensitive to sample pretreatment and more representative of APS produced by microorganisms in marine environments than XG. Hung et al. [73] also evaluated the use of carbohydrate measurements as an alternative to dry weight measurements for the calibration using standard polysaccharides (compare with the TOC measurements done by Villacorte et al. [151]). Moreover, alginic acid has an anion density of almost 1 carboxyl group per monosaccharide, enabling the easy conversion of alginic acid equivalents to carbon concentrations. It is worth noting that there is again some terminological inconsistency: Hung et al. [73] classified the TEPs measured by the Passow and Alldredge (1995) method as APS.

Importantly, a comparison was made with TEP concentrations obtained by

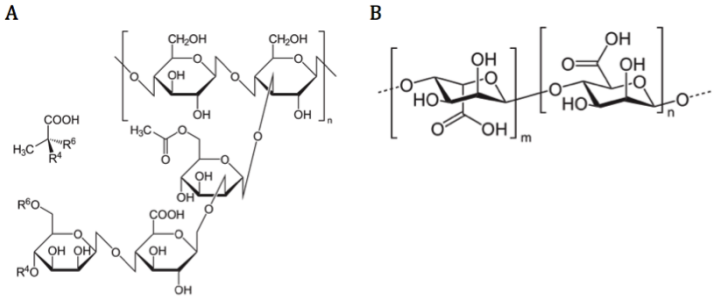

Figure 1.4: (A) XG; (B) Alginic acid.

the Passow and Alldredge (1995) method to investigate the dynamics between particulate TEPs and soluble APS. Arruda Fatibello et al. also compared their own method (which would be expected to measure a fraction similar to APS) to that of Passow and Alldredge (1995), and found that the TEP concentrations found by both methods were more or less the same. Thornton et al. [135] obtained very different results: in seawater samples APS concentrations (Thornton method) were consistently greater than TEP concentrations obtained with the Passow and Alldredge (1995) method. This could be expected since APS are a pool of acid polysaccharides that includes both the particulate (TEP > 0.4 μm), colloidal and the dissolved (>1000 Da) AB stainable sugars. In microalgae broths, Thornton et al. [135] found TEP concentrations consistently being higher than APS concentrations because colored substances from cell interiors lead to an over-estimation of TEP concentrations. The other freshwater samples contained TEP concentrations that were not significantly different from whole water APS, and indeed had a lower proportion of the APS passing through a 0.4 μm filter.

Another test was performed to examine what the APS are exactly and how they relate to the fraction measured by the established TEP assay. A comparison was made between regular water samples (freshwater containing a dense bloom of chlorophyte) and water samples that had been processed using a cell disruptor. Cell disruption only slightly increased the mean concentration of both TEPs and APS. This indicated that both methods (the Passow and Alldredge (1995) and the Thornton method) result in a substantial release of intracellular APS in the whole water samples, probably due to the low pH of the assays. The combination of sample storage in formaline or desalting by dialysis could compound the release. Therefore, to prevent ambiguity, the authors advised disrupting the cells via sonication or mechanical breaking before staining to ensure the full APS staining.

The results found by Thornton et al. [135] imply that the TEPs quantified by the established method may also over-estimate the TEPs if organisms have AB stainable substances bound to their cell wall. Passow and Alldredge believed that only limited material, if any, could leak from the cells and subsequently be stained by AB due to the low filtering pressure and brief exposure to AB solution (2 seconds). However, both impact on the filter and exposure to low pH could result in the liberation of significant AB staining material and subsequent over-estimation of TEPs. Like with the Thornton method, cell integrity is likely to be compromised by the combination of formalin addition to preserve samples, the reduction of pH to 2.5, filtration, and dialysis.

For ecological research, but also for membrane fouling research, it is important to be able to distinguish between internal and external pools of APS. According to Thornton et al. [135], this does not seem to be possible by use of either assay on whole water samples. However, by removing cells using gentle filtration before performing analysis, it is possible to get an accurate measure of APS in the dissolved pool.

1.4.3 TEPs: 'Sticky particles that associate with magnetic microspheres'

Mari and Dam [94] developed a method in an attempt to eliminate the use of AB for staining TEPs. In contrast to the Passow and Alldredge, Aruda Fatibello and Thornton methods that give quantitative measurements of TEP in bulk solution, the Mari and Dam method merely detects, isolates and concentrates TEPs and cannot be used to quantify TEP concentrations, although this initially seemed to be the purpose. They proposed stickiness as quantification parameter, which gives direct information about the adhesion potential of these compounds upon collision. The stickiness property is very relevant in relation to their role in biological processes, like aggregation/sedimentation (and, for that matter, for their role in membrane fouling), especially because it is known that TEP stickiness is much higher than that of cells and 2 to 4 orders of magnitude higher than that of most marine particles [49, 114]. The method consists in adding micrometer-size paramagnetic microspheres to a bubbling column together with the TEPs. Bubbling causes the TEPs and microspheres to form mixed aggregates. After this, TEP staining with AB and microsphere enumeration is performed. The authors found that all TEPs formed complexes with the microspheres and the microspheres never aggregated by themselves by means of a biological glue insensitive to AB staining (no clusters of microspheres alone with a ghost matrix). In other words, AB and the microparticles detect the same fraction. The microspheres have been proven useful for some applications,

like the influence of metal concentrations on the stickiness of TEPs [96] and the influence of ageing on the stickiness of TEPs [125].

1.4.4 Other determination methods

Double staining methods

There have been several indications for the colonization of microgels by bacteria [145], and double-staining methods have been successfully used to examine bacterial colonization of TEP. Logan et al. (1994) first showed that samples stained with AB can be simultaneously stained with fluorochromes like 4,6-diamidino-2-phenylindole (DAPI). By using AB and DAPI in combination, Mari et al. observed that all TEPs in their samples were colonized by bacteria (in their study the TEPs were obtained by filtering through 0.2 μm filters) [93]. Berman et al. used SYBR Green with AB to study the morphological properties and bacterial colonization of TEPs (obtained with 0.4 μm filters) [21].

Different dyeing materials: other transparent species

TEPs are not the only kind of microscopic transparent organic particles present in marine and freshwaters. There are other particles stained by DAPI as described by Mostajir et al. [102]. Long and Azam [90] described a class of protein-containing, transparent particles in seawater upon staining with Coomassie Blue: CSPs (Coomassie stained particles). Bar-Zeev et al. [16] pointed out that it is quite possible that some, if not most, of these transparent organic particles contain varying amounts of polysaccharide, protein and/or nucleic acid constituents. Heinonen et al. [65] also mentioned the use of Coomassie Blue to stain exopolymers. However, several studies showed that these stains target different fractions of exopolymers: CSPs have been found either to be more abundant than TEPs (Long and Azam, 1996), or similar in abundance [60] or less abundant [119]. A careful comparison of both particle types in Lake Kinneret revealed that there were, on average, more TEPs than CSPs throughout the year. The latter presumably have faster turnover rates [22]. In other word, the transparent particles may consist of other materials than cannot be stained by AB.

Fluorescent labeling of specific carbohydrate moieties

Fukao et al. [55] examined the properties of TEPs with the use of fluorescently labeled lectins: FITC-concanavalin A, which is specific for D-mannose and/or

D-glucose, and Ulex europaeus agglutinin-1-FITC, which is specific for L-fucose. This was done because the composition of the TEPs may determine the viscosity of the marine mucilage (TEPs with high L-fucose content are known to be highly viscous) [55]. Uthicke et al. [137] developed a method using the lectins and a 96 well plate to quantify particulate polysaccharides more broadly. The lectin-based method is claimed to be a cost-effective and rapid complement to the AB based methods. In addition, it requires significantly smaller sample volumes. The use of a diverse set of lectins that specifically stain different polysaccharides may provide specific insights to the full spectrum of marine polysaccharides and also provide clues about the sources [137] .

Other techniques used to determine nano- and microgels

Techniques used to determine nano-and microgels, such as flow cytometry and dynamic laser scattering spectroscopy (DLS) may also be relevant to study TEPs [146]. Flow cytometry provides optical measurements of individual cells or particles by suspending them in a stream of fluid and passing them by an electronic detection apparatus with a tightly focused laser beam. The laser light is scattered and induces fluorescence signals that give characteristic optical signatures for different cells or particle types. In oceanography, flow cytometers have been used to study marine biopolymers [32, 107, 108, 147]. Modern flow cytometers can be used to separate marine microgels from a heterogeneous mixture like seawater for further analytical analysis. In the study of Orellana et al. [108], a high-speed cell sorter was adapted to interface with a scanning monochromator and this instrument was used to characterize optically active marine microgels from native seawater, as well as those produced by phytoplankton cells in culture. In the study of Orellana et al. [107], flow cytometer measurements of microgel forward scatter were used to monitor changes in volume. DLS is a method for determining the size distribution profile of small particles in suspension or of polymers in solution. DLS yields reliable sizing in the submicrometer range [32], and has been used in different studies for the monitoring of the assembly of dissolved organic matter polymers [32, 44, 147]. The Stokes radius, dynamic conformation, and polydispersity of free polymers and colloidal and particulate material can be studied by DLS [145]. The application of flow cytometry and DLS offers great potential for the detection and quantitative assessment of microgels concentrations in seawater [146].

Other methods

Claquin et al. [35] adapted the Passow and Alldredge (1995) method for the determination of TEP concentration and its expression in XG equivalents per liter (XG/L), incorporating centrifugation (instead of filtration), like in the Arruda Fatibello 2004 method. 5 mL of culture was centrifuged at 4000 rpm (3200 × g) for 20 min. 2 mL of a 0.02% AB (Sigma) and 0.06% acetic acid solution was added to the pellet. The sample was centrifuged (3200 × g, 20 min) immediately in order to remove the excess dye. The pellet was rinsed with 1 mL of distilled water and centrifuged several times until excess dye was totally removed. 4 mL of 80% sulfuric acid was then added to the pellet. After 2 h, the absorption of the supernatant was measured at 787 nm. This method presumably detects a completely different fraction than the other methods. Instead of detecting the soluble fraction, it detects the fraction that stays with the cells after centrifugation. It subjects the cells to the pH 2.5 AB solution, after which sulfuric acid is added to the cells and the absorbance of the whole is measured. As pointed out by Thornton et al. [135], the cells can be broken by submission to low pH. Furthermore, measurement of absorbance at 787 nm can also measure cellular components other than AB. In conclusion, a large proportion of what is measured here, apart from TEPs, are probably internal cell components.

1.5 Classification of TEPs: unifying the terminology

As mentioned above, different methods to quantify TEPs mostly measure different fractions, especially regarding their size. In what follows, the methods are grouped according to the definition of TEPs they imply. The use of the same terminology for different materials often creates confusion. Here we try to map all materials stained and measured by all established methods and propose terms for different fractions (see Figure 1.5). As discussed in Section 1.3, AB selectively stains sulphated glycosaminoglycans, glycoproteins, hyaluronic acid and glycoproteins that owe their acidity to sialic acids [80]. Therefore, it is more precise to group them as 'stainable materials by AB at pH 2.5'. However, it is worth noting that other materials not stainable with AB also exist in transparent particles, as discussed in Section 1.4.4. AB-stainable materials may exist in different sizes: particulate, as defined by Passow and Alldredge (1995), when the size is bigger than 0.4 μm); colloidal, as proposed by Villacorte et al. [151], when they pass through 0.4 μm filter and are retained by 0.05 μm filter. When smaller than the latter, they are present as a solution. Figure

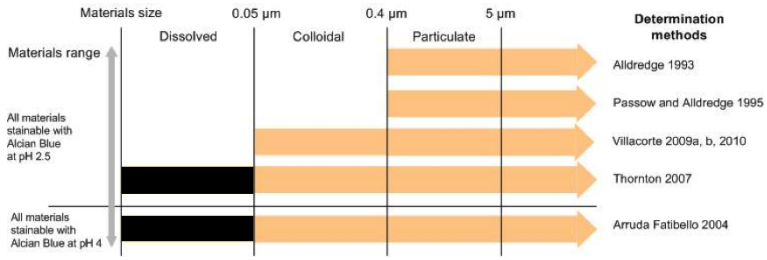

Figure 1.5: The range of possible materials determined by the different methods discussed in Section 1.4. Two main parameters can be used to classify them: size and pH. Other methods, not included in this figure, are rather more specific; i.e, Mari and Dam [94] measure the stickiness properties, Fukao et al. [55] measure the most viscous fractions. (Remark: according to the IUPAC definition, dissolved material is material from 0.001-1 µm, but filters with pore sizes smaller than 0.05 µm cannot be used.

1.5 presents what exactly is measured by the different determination methods. However, since there is no clear justification on what materials are selectively stained by AB at pH 4, it is hard to distinguish what actually is measured by the Arruda Fatibello method (see Figure 1.6). However, when pH 2.5 would be adopted, it most likely measures a similar fraction as the Thornton method. To decide which method should be used in a certain situation, several factors should be considered: (a) the concentration of TEPs in the sample (the Passow and Alldredge test is still the most sensitive) (b) the wanted precision (the Passow and Alldredge test is still the most accurate) (c) if it is a fresh water or sea water sample (salts could interfere with the Arruda Fatibello method) (d) the amount of samples to be measured and the amount of sample (the Arruda Fatibello method is the fastest method and does not require a large sampling volume).

1.6 TEPs and membrane fouling

TEPs have been the suspects of membrane fouling since 2005, when Berman and Holenberg indicated some important properties that make them likely culprits, such as their extreme flexibility and stickiness. Since then, TEPs have been monitored in a number of membrane settings, but the way they were determined differs [20, 41, 153]. One remaining question is which TEP determination method is best suitable for membrane fouling research. What are the properties of TEPs that are most important for their role in biofilm

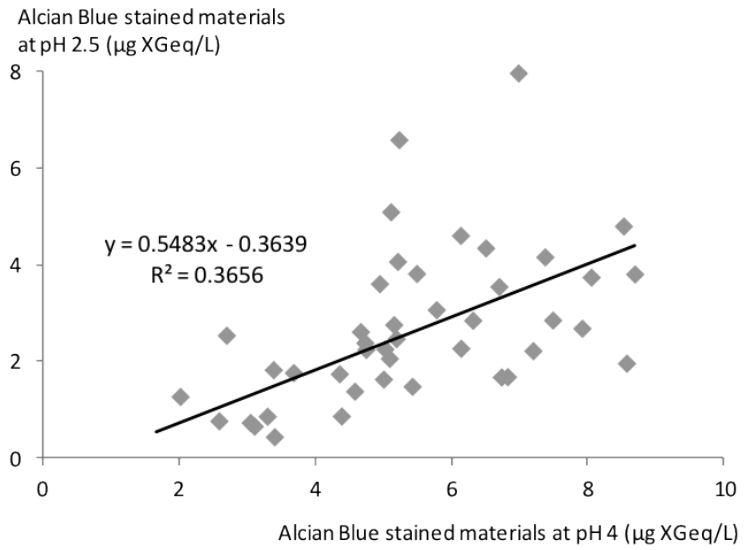

Figure 1.6: Correlation between stainable materials by AB at pH 4 and 2.5, measured for *Chlorella vulgaris* broth according to Arruda Fatibello method at corresponding pH. The slope (less than 1) indicates that the amount of stainable material at pH 4 almost doubles the one at pH 2.5.

formation on membranes or colloidal fouling initiators and to what extent are those measured in a different way by the different methods? As mentioned above, the methods differ in the size class of the TEPs measured (soluble, colloidal, particulate) and the composition (stained by AB at pH 2.5 or pH 4; stained by fluorescent lectins; determined by paramagnetic nanoparticles). Firstly, the size seems to be important. In a recent article, Bar-Zeev et al. [14] mentioned that GF/F (glass fibre, class F) filtration, which effectively removed the >2 μm TEPs (26-47% of the total amount of TEPs) and other large particles, slows down the initial phases of biofilm formation in a flow cell. This highlights the importance of these microgel particles in facilitating the initial phases of biofilm formation. These experiments also showed that even when large TEPs and protobiofilms (TEPs with extensive microbial outgrowth and colonization) were removed from the overlying water, early biofilm could still develop, albeit at a much slower rate [14]. In the studies of Villacorte et al., the fate of the particles in membrane systems was investigated. In one study, both pTEPs and cTEPs were present in the UF permeate [153], which was not the case in their previous study where pTEPs were totally removed by UF [151]. The membranes in these studies all had a nominal pore size of 30 nm. In the study

of Van Nevel et al. [139] UF membranes were investigated with a pore size of 100 nm. Since TEPs are known to be highly flexible, they can pass through membranes with a nominal pore size smaller than their own diameter, especially when high pressure is applied. Surprisingly, the membrane retained cTEPs, with diameters varying from 0.05 to 0.4 μm successfully. This would suggest that the majority of this fraction were particles with diameters between 0.1 μm and 0.4 μm. Thus, it seems to be of importance to be able to distinguish in TEP sizes. This can be easily done by implementing filtration steps prior to applying the Arruda Fatibello method, but the question is then whether the method is still more rapid and efficient than the Passow and Alldredge (1995) method. The size of the TEPs is however not a static property. Smaller sized TEPs can coagulate in turbulent environments to form larger sized TEPs [139, 146]. Therefore, the importance of the size will also depend on the set-up investigated: with cross-flow (more turbulence) or dead end; microfiltration (lets most small particles through), reverse osmosis (retains even the smallest organics). Also the composition of what is measured by different methods probably differs because of the different pHs that are used. The difference probably mainly depends on the sample. Indeed, a calibration with XG should give the same results for pH 4 or 2.5: the carboxyl groups on XG will be negatively charged at both pHs. This also explains the results of Worm and Søndergaard [157]. They stained particles with an AB solution that was not acidified, and called them ABSP (AB stained particles). They compared the staining procedures and found that the ABSP were 44% more abundant than TEPs, but also that the difference was not statistically significant, indicating that most ABSP were likely to be TEPs. On the other hand, if it is the influence of particles on membrane fouling that is to be considered, maybe the particles that form complexes with AB at a pH $\leq$ 2.5 do not have to be the only ones considered. Furthermore, it has been mentioned before that TEPs are most likely associated with other components like nucleic acids, proteins and bacteria [12, 19, 21]. Maybe the particles that are negatively charged at pH 4 are the real culprits of membrane fouling. This could be the case in a study of de la Torre et al. [42, 41], where the critical flux values correlated with four parameters (temperature, nitrate, bound and soluble TEPs) measured in the activated sludge for 95% of the data. TEPs were measured at pH 4 with the Arruda Fatibello method. However, no comparison was made with samples stained at pH 2.5. Furthermore, de la Torre et al. were dealing with bacterially derived TEPs, which likely contain different functional groups, which can react differently with AB.

1.7 Conclusions

Several methods are available for TEPs determination, which often determine different fractions, both in material composition and size. As they all still apply the same terminology, confusion remains and difficulties arise in comparing results from different studies. The TEPs measured by the different methods were mapped (Figure 1.5) to keep a clear view on which fraction is measured and how one relates to others. When adopting an established method, it is strongly recommended to describe in detail any changes to provide clarity on the measured material. The TEP size is crucial in a membrane fouling context, since fouling mechanisms are different in different membrane filtration processes (membrane bioreactors, reverse osmosis, microfiltration, cross flow, dead-end).

Chapter 2

TEPs and membrane fouling in a full-scale ultrafiltration plant: feed parameters analysis and membrane autopsy

Adapted from: Discart, V., Bilad, M. R., Van Nevel, S., Boon, N., Cromphout, J., and Vankelecom, I. F. J. Role of transparent exopolymer particles on membrane fouling of a full-scale ultrafiltration plant: feed parameters analysis and membrane autopsy. *Bioresource Technology* 173C (2014), 67-74.

Abstract

Ultrafiltration (UF) is widely used for water purification, but membrane fouling remains an important issue. In this study, the role of transparent exopolymer particles (TEPs), recently put forward as possible major foulants, was investigated in the fouling process of a full-scale UF installation. Algae, TEPs and other parameters in the UF feed were monitored and correlated during an 8 months long full-scale operation. Results revealed a complex fouling mechanism involving interactions mainly between algae, Fe (flocculant) and

TEPs. Algae related parameters rather than TEP concentrations correlated stronger with irreversible fouling rates, suggesting that the overall role of TEPs in membrane fouling seems limited for this application. Finally, membrane autopsy showed the formation of a thick Fe-rich fouling layer on top of the fouled membranes, which was largely removed part by cleaning-in-place. Part of it was irremovable due to the formation of Fe-organic complexes.

2.1 Introduction

TEPs are transparent and sticky gel particles that are ubiquitous in natural waters. They are primarily formed from polysaccharides excreted by microalgae [113]. Several recent studies indicate a potentially important role of TEPs in the biological and colloidal fouling of membranes during filtration [153, 152, 151, 20, 15]. Their influence was studied in a wide variety of membrane processes, including reverse osmosis [152, 153, 15], ultrafiltration (UF) [20, 150], and membrane bioreactors [41]. In all these systems, TEPs were found to contribute to membrane fouling.

For UF membranes in particular, TEPs were proven to cause irreversible membrane fouling [153]. Therefore, Bar-Zeev et al. suggested that the measurement of TEPs in different stages of pretreatment before UF or reverse osmosis filtration could be an effective means for the optimization of membrane filtration of wastewater and seawater [15]. Efforts have already been made to deal with the impact of these particular materials, not only by membrane installation operators, but also by membrane providers. Some membrane providers (i.e., Sumitomo Electric Industries, Ltd.; Ahlstrom Corporation) have started to broaden their focus from classic water quality parameters, (e.g. the silt density index) to TEPs to optimize membrane performance. Investment in the development of TEP captation mechanisms seems to emerge: Sumitomo Electric Industries, Ltd., has developed a filtration membrane, named TEP Trap membrane, which can efficiently trap TEPs in the pretreatment step, and a TEP Trap apparatus which incorporates a washing mechanism to blow TEP away from the TEP Trap membrane [131].

The impact that TEPs could have with respect to membrane fouling during the filtration of surface water (i.e., for the production of drinking water) is immense. The surface water usually comes from an open pond or stream, which allows the growth of microalgae. Microalgae are the main producers of TEPs in natural environments [113]. In our recent study, TEPs were proven to be a part of algogenic organic matter (AOM) of *Chlorella vulgaris*, mostly produced during growth [46]. So as long as microalgae can grow in the feed reservoir, the presence of TEPs is inevitable.

Few reports exist that deal with the influence of TEPs on membrane fouling in a full-scale membrane filtration plant for the production of drinking water. Van Nevel et al. (2012) identified the presence of the TEPs at different stages of drinking water treatment but did not relate it to the performance of the membrane filtration [139]. In the present study, the relation between feed properties, with an emphasis on TEP related parameters, and UF membrane fouling for surface water filtration in a full-scale plant was investigated. The full-

scale plant (operated by the Flemish Water Supply Company De Watergroep, and located in Harelbeke-Stasegem, Belgium) uses surface water as a feed source (taken from the canal Bossuit-Kortrijk which is fed by the Scheldt river). The river is polluted by industrial, municipal and agricultural waste, which thus requires an extensive pre-treatment, consisting of, among others, direct filtration over 3 layer pumice/anthracite/sand filters.

In this installation, several measures are incorporated to limit fouling during the dead-end UF, such as backwash, chemically enhanced backwash (CEB) and cleaning-in-place procedures (CIP). Also, in-line coagulation by dosing a small amount (3 mg/L) of flocculant (iron chloride) is applied to avoid pore blocking. However, even after such treatments, irreversible membrane fouling on the long term was observed. This fouling was hypothesized to result from the TEPs present in the water source.

In this study, the origin and the behavior of TEPs through this installation were considered. Firstly, the algae and TEP concentrations in the UF feed water were monitored over an 8 months period. The association of the feed parameters to the filtration performance was evaluated using Pearson Coefficient correlation analysis to distinguish the most important parameters and their degree of association with membrane fouling. Finally, two smaller UF membrane sample modules were installed in one of the racks of the full-scale installation to allow a membrane fouling autopsy study towards the end of the observation period. The autopsy was performed after approximately 5 months of operation with the first sample (fouled condition) taken out just before the next CIP, and the second sample (cleaned condition) just after CIP.

2.2 Materials and methods

2.2.1 Overview of the water purification plant

Design

A full description of the purification plant is given by Cromphout et al. [37]. In short, the raw canal water is first sent through a pretreatment to remove ammonia and phosphate. This pretreatment consists of oxidation in aerated reactors, and of coagulation with iron (3 mg Fe/l), flocculation, and direct filtration over three-layer filters (Figure 2.1). After the pretreatment, the water is stored in an artificial lake “de Gavers” with a storage capacity of 3.2 Mm^3. From the lake, the water is reintroduced into the treatment plant and post-treated by coagulation-filtration (identical to the coagulation-filtration step

used for phosphate removal in the pre-treatment), by activated carbon filtration and chemical disinfection. In 2009, the production capacity of the conventional drinking water production plant was extended from 25,000 to 32,000 m^3 d^{-1} by the construction of an UF unit in parallel with the second coagulation-filtration step. The UF unit consists of four independent racks (skids), each with 40 vertically placed polyethersulphone (PES) membrane modules. The racks are designed in such a way that modules of different origins can be placed. One rack contains modules with multibore capillary membranes with an internal diameter of 0.9 mm, with a total membrane surface of 2000 m^2. The other three racks contain single bore capillary membranes with an internal diameter of 0.8 mm, and a surface area of 1600 m^2 per rack. The capillary membranes are all used in inside-out filtration direction modus. Every module has two feed/concentrate connectors (above and below) and one permeate connector [37]. Since the filtration was performed in dead-end mode, concentrate pressure is referred here as the pressure of the opposite end of the feed entrance.

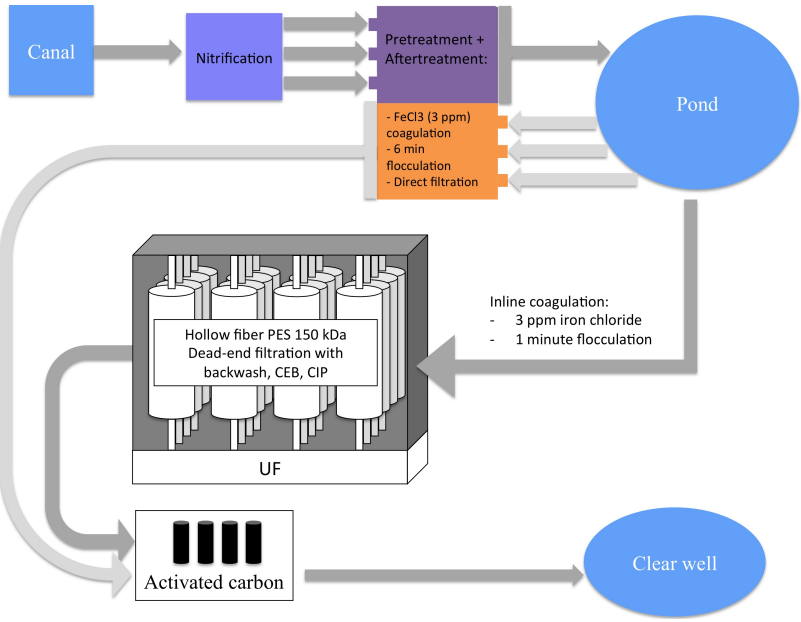

Figure 2.1: Scheme of the full-scale process

Several measures are taken to limit fouling. A backwash is performed after every filtration cycle of 60-90 min. The filtrate direction is alternated from top-bottom to bottom-up or vice versa after each filtration cycle, and backwash is performed counter current with respect to the preceding filtration run. Every

30 cycles, a CEB is performed by dosing sodium hydroxide or sulphuric acid in the backwash feed pipe, optionally combined with hydrogen peroxide. A CIP is applied only when the transmembrane pressure (TMP) at the end of the filtration runs exceeds 350 mbar. At the start of this study, in April 2012, three to four CIP's with oxalic acid were necessary every year [37].

Characterization of fouling

To monitor the UF process, each rack is equipped with online pressure measurement devices, which measure the pressure in the feed, the concentrate and the permeate. Based on these online measurements, the TMP and membrane permeance are calculated every 5 min using Eqs. 2.1 and 2.2:

$$TMP = \frac{P_f + P_c}{2} - P_p \tag{2.1}$$

$$L = \frac{Q_f}{A.TMP} \tag{2.2}$$

where P_f, P_p and P_c are pressure in the feed, permeate and concentrate collector, respectively. L is permeance (L/m^2 h bar), Q_f feed flow rate (L/h) and A membrane surface area (m^2).

The irreversible fouling rate (IFR) is defined as permeance loss that can be recovered by the applied chemical cleaning (removal by CIP), while reversible fouling is defined as the permeance loss that can be recovered by physical measures (such as backwashes or CEB's), and irrecoverable fouling is the permeance loss which remains even after CIP [47]. From the filtration data, two fouling parameters were calculated as a measure for the irreversible fouling rate to evaluate the filtration performance. First, the decrease in permeance during four days before and four days after TEPs measurement was taken as the IFR_L. Secondly, the rise in filtration resistance (R, 1/m) over the same time periods were determined (further referred to as IFR_R), with resistance calculated based on Eq. 2.3:

$$R_F = \frac{TMP}{\eta J} = \frac{1}{\eta Perm} \tag{2.3}$$

where η is the dynamic viscosity (Pa s) and J the flux (L/m^2 h). By means of the dynamic viscosity a correction for temperature variations is implemented.

The two parameters were compared with each other, for the different racks and with the different feed water characteristics.

2.2.2 Monitoring of the feed water parameters

Over 25 water parameters, including chlorophyll and multivalent ions, were routinely measured in the canal and pond water. In addition to those parameters, TEP concentrations in the pond were determined in duplicate weekly or biweekly according to the Passow and Alldredge method [115], with modifications regarding the filter pore size as suggested by Villacorte et al. [153]. In short, between 10 and 50 mL of sample was filtered over a polycarbonate (PC) filter (0.4 µm for particulate or pTEPs and 0.1 µm for colloidal or cTEPs), after which the filter was stained with 1 mL of a pre-filtered solution containing Alcian Blue (AB) and acetic acid at pH 2.5. The AB concentration of the dye solution was always determined before staining by measuring the absorbance at 787 nm and adjusting it to a value of 0.150 to obtain a constant stain concentration of 150 mg/L[46]. Xanthan Gum (XG), a commercially available polysaccharide, was used as a model for TEPs in the measurements and therefore TEP concentrations were expressed as mg/L XG equivalents (mg XG/L). The calibration for this staining method was performed by (total organic carbon (TOC) measurements, as explained by Kennedy et al. [79] and yielded a calibration factor f_x of 327 mg XG per unit absorbance at 787 nm. Chlorophyll concentrations were obtained by extraction, and optical density measurement. Briefly, $MgCO_3$ 0.1% was added to the water sample. After that, the water sample was filtered through a membrane (cellulose nitrate Millipore AAWPO4700 with pore size 0.8 μm. The membrane is then placed in a glass centrifuge tube and aceton 90% is added. The centrifuge tube is placed in an ultrasone bath during 20 min to let the extraction process take place. After the extraction, the centrifuge tube is centrifuged during 20 min at 3000 rpm. Consequently, the supernatant is put in a cuvette (4cm) and the optical density is measure d at 4 different wave lengths (630, 645, 665 and 750 nm). After this, 3 drops of HCl 1 N is added to stimulate the conversion of chlorophyll to pheophytine. The optical density is measured again at wavelengths 665 and 750 nm.

2.2.3 Sample modules: characteristics and autopsy

Two sample modules were placed in rack 4, starting on the 17th of July 2012 until the 11 and 12th of December 2012. The small modules contained capillaries of the same type as the large modules and installed after a CIP-procedure. One was taken out for autopsy just before and another just after the next CIP, to

get a fouled and a cleaned sample. The modules contain multibore membranes similar to the full-scale ones.

Autopsy: membrane sample preparation

For some characterization tests, the fouling substances had to be taken off the membrane. Scraping was not possible because the fouling layer was firmly attached to the membrane. Therefore the membranes were placed in an oxalic acid solution of 5 g/L and placed in a sonication bath for 15 min. Oxalic acid is normally used for CIP in the full-scale installation. For direct observation of the membrane surface, the 7-bore membranes were cut open.

Characterization of solubilized foulants

After the solubilization, the foulants were subjected to a TOC-analysis (Analytic MultiNC), calculated as the difference of total carbon (TC: TCcalibration NPOC60) and total inorganic carbon (TIC: TICcalibration NPOC+). They were also subjected to an atomic absorbance spectroscopy for the potassium, calcium and sodium concentrations as well as inductively coupled plasma spectroscopy for iron, aluminium and magnesium concentrations.

Morphological characterization

The membrane surface microstructures of the pristine membrane, the fouled membrane after filtration, and the cleaned membrane after CIP were observed via scanning electron microscopy (Philips SEM, XL30 FEG, with EDX dx-4i system). The membrane samples were subjected to a fixation procedure with 4% paraformaldehyde, as described by Declerck et al. [43]. After fixation, the membrane SEM samples were dried and coated with a sputtered gold layer prior to analysis.

Component characterization

Surface analysis of (mostly metal) elements was conducted with Energy-dispersive X-ray spectroscopy (EDX) analysis, integrated in the SEM (see above) [120]. This measurement was applied to identify fouling by inorganic elements. For detection of organic material, the membrane was analyzed with ATR-FTIR (Attenuated total reflectance-Fourier transform infrared spectroscopy - Bruker, Alpha) after drying for 24 hours in ambient conditions.

2.2.4 Statistical analysis

A Pearson regression coefficient (r) was used to define (1) the correlation among feed water parameters and (2) the correlation between individual parameters and the IFRs. The Pearson regression coefficient is normally used to express the intensity of a linear relationship between a pair of parameters. Values are between -1 and 1: the closer the values to ± 1, the stronger their relationship. Minimum r values are required to fulfill a certain degree of significance, also as a function of sample number. For the different samples used in this study, the degree of correlation between the tested parameters was defined as low significance for a 95% confidence level, medium significance for 98% confidence level and high significance with 99% confidence level. They are marked with asterisks *, ** and *** respectively for low, medium, and high significance levels, and with brackets to indicate if there is a negative correlation.

2.3 Results and discussion

2.3.1 Water characteristics and filtration performance

Water characteristics

All water parameters, except TEP concentrations, were followed in the pond, the canal, and in the UF filtrate. TEP concentrations were only followed in the pond (which contains the direct UF feed water), and the correlations were made with both the algae-related-parameters in the canal and in the pond. This was done to understand the presence and the origin of TEPs in the feed water. The TEPs and algae-parameters evolution during the test is presented in Figure 2.2. It should be pointed out that TEP concentrations found in this study are somewhat low in comparison with those found in other studies (Table 2.1). As in other studies where this portion was considered, cTEPs composed a significant portion (30-95%) of the total TEP concentration. In general, it seems that TEP concentrations are generally lower in temperate environments than in Mediterranean regions [117].

Since microalgae are known to be the main producer of TEPs, the abundance of TEP is expected to have a strong association with the abundance of microalgae. As shown in Figure 2.2, the connection between TEPs and pond algae parameters was surprisingly not very clear, possibly due to the relatively low concentrations, thus vulnerability to measurement errors. The $\text{chla}^{\text{POND}}$ concentrations were never higher than 25 µg/L (see Figure 2.2 A and B). These low values are due to

Table 2.1: Overview of TEP concentrations in literature and this study (XG = Gum Xanthan equivalent; "/" = no measurement)

Sample type	pTEPs (μg XG/L)	cTEPs (μg XG/L)	Reference
Literature			
Seawater (California)	3-310	/	[115]
Seawater (Mediterranean Sea)	230-478	/	[14]
Seawater (Gulf of Mexico)	100-4500 #		[135]
Coastal seawater (Netherlands)	400-800	800-4100	[153]
Secondary wastewater effluent (Belgium)	102	1470 *	[139]
Surface water (Kluizen, Belgium)	14.8	684 μg *	[139]
Ground water (Belgium)	<5	<50 *	[139]
River water (Meuse, Netherlands)	270	250	[152]
Surface water (the Netherlands)	990		[79]
Surface water (Israel)	759-2385		[21]
This study			
Surface water in pond de Gavers	2-143	5-137	

*calibration factor determined with TOC measurements
#Thornton method measured on the whole water sample (without filtration)

the pretreatment of the water before entering the pond, which removes most of the nutrients necessary for algae blooms. Both in the pond and the canal, chla clearly constituted the largest portion of the total chlorophyll concentrations, while the chlb concentration was very low. Pheophytine (Pheo) concentrations always followed total chl concentrations very strongly.

To determine correlations between the different feed (pond) water parameters, a statistical analysis was performed (see Table A.1 and A.2 in the appendix for all correlations). This way, the sample values obtained from the canal and the pond could be correlated. Also, since the main objective of this study was to investigate the impact of TEPs, most emphasis was given to link TEP concentrations to other parameters. It should be noted that the hydraulic retention time in the lake is around 100 days (3.2 Mm^3/32,000 m^3 d^{-1}), so the correlation between the canal and UF feed water variables would be expected

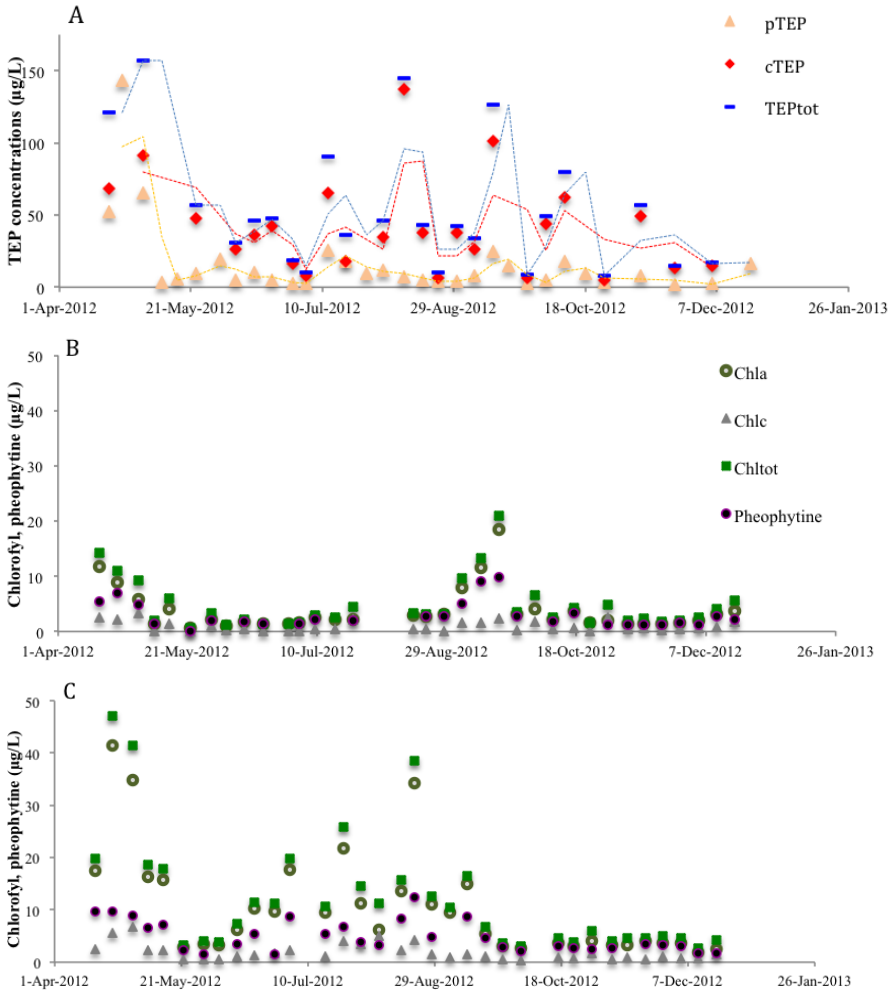

Figure 2.2: (A) TEP concentrations in the pond (pTEP = particulate TEP; cTEP = colloidal TEP; TEPtot = total TEP); (B) Algae parameters in the pond; (C) Algae parameters in the canal (Chla, Chlc and Chltot = chlorophyll a, chlorophyll c and total chlorophyll).

to be zero in circumstances of perfect mixing. This expecially since there are pretreatmetns that would alter the canal water before it reaches the pond.

As shown in other studies [113, 54, 148, 16, 35], a connection between algae

parameters (canal and pond concentrations) and TEPs (only measured in the pond) was found. The most important parameters, screened from the results among 23 parameters in Table A.1 and A.2 of the appendix are summarized in Table 2.2: both cTEPs (nominal size size between 0.1 and 0.4 µm), and total TEPs correlate strongly with $chla^{POND}$, $chlc^{POND}$ and $chltot^{POND}$. The pTEPs correlate strongly with $chlc^{POND}$ and $chla^{CANAL}$, $chlc^{CANAL}$ and $chltot^{CANAL}$. This link between pTEPs and canal parameters is surprising (see above), and could be explained in several ways. One possibility is that there is an extremely imperfect mixing which causes water coming from the pretreatment to go almost directly to the UF. In that case, these results suggest that the pTEPs were derived from algae in the canal, possibly due to coagulation of the cTEPs in the pretreatment (consisting of nitrification and direct sand filtration), while the algae and initial large TEPs were retained. Villacorte et al. showed in their study that TEPs, due to their flexibility can indeed evade pretreatment consisting of coagulation/sand filtration [152]. Another interesting correlation was the one between chlc in the canal and chlc in the pond, which indicates that chlc (which is smaller than chla or chlb) from canal algae passes through the pretreatment. Of the other parameters, only the suspended solids (SS) correlated significantly with both the TEP parameters and the algae parameters. In general, pond algae seemed to be the producers of TEPs in the pond, and high SS could be an indication of high TEP concentrations.

Filtration performance

The permeance profile of the 4 racks is shown in Figure 2.3. Both membrane fouling parameters IFR_L (calculated as the permeance loss) and IFR_R (calculated as the rise in filtration resistance) were later linked via the Pearson coefficient correlation to all feed parameters, as shown in Table A.3 and A.4. This way, parameters that are closely associated with membrane fouling can be sorted. Also, the similarities or differences between the racks can be evaluated. The summary of these most important feed parameters is presented in Table 2.3. The IFR_L and IFR_R data from different racks were used separately. These feed parameters were suspected to play an important role in membrane fouling.

The overall profiles of permeance were similar for rack 1, 2 and 3, but not for rack 4 (which contained the sample modules). Even though all racks were started at the same time, rack 1, 2 and 3 underwent a CIP in the middle of the study period, and rack 4 at the end of the study. This can be explained by the type of applied membranes. Racks 1, 2 and 3 contained single bore type membranes, while rack 4 contained multibore membranes. This shows the advantage of multibore over single bore membranes. Racks 2 and 3 appeared to show a very similar membrane fouling behavior (Table A.3), but different from

Table 2.2: Correlations between feed water parameters

		Pond								Canal		
		pTEP	cTEP	TEP_{tot}	Chla	Chlc	Chltot	Pheo	SS	Chla	Chlc	Chltot
Pond	pTEP	1										
	cTEP	***	1									
	TEP_{tot}	***	***	1								
	Chla	*	***	***	1							
	Chlc	***	***	***	***	1						
	Chltot	**	***	***	***	***	1					
	Pheo	*	**	**	***	***	***	1				
	SS	**	***	***	***	***	***	***	1			
Canal	Chla	***							*	1		
	Chlc	***	***	***		***			*	***	1	
	Chltot	***				***			*	***	***	1
	Pheo	*						*	*	***	***	***

*** : p<0.01; ** : p<0.02; * : p<0.05; TEPtot = total TEP concentration

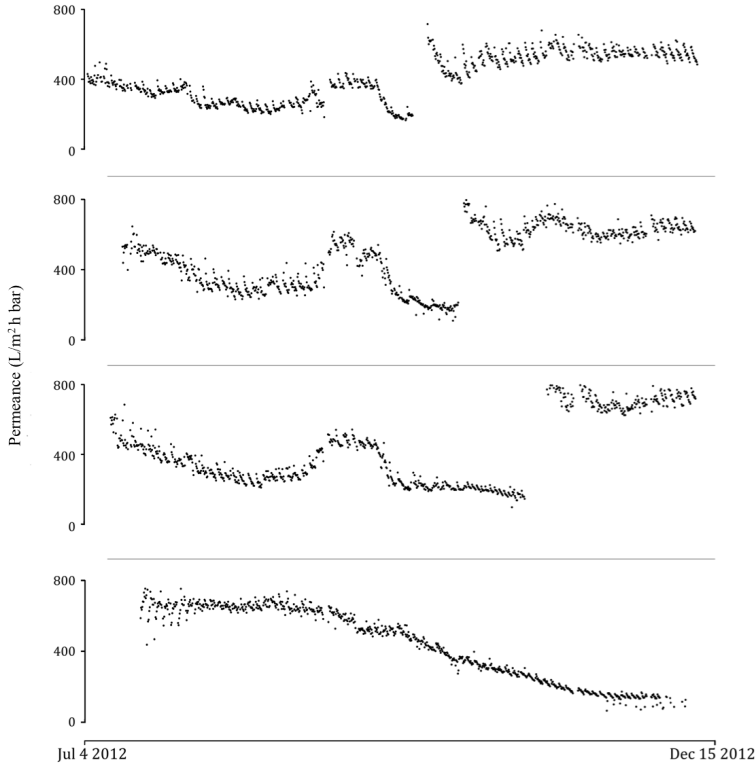

Figure 2.3: Evolution of permeance of the four racks over the testing duration (rack 1 is shown in the top graph, rack 4 in the bottom graph). The rate of permeance loss (IFR_L) is calculated as the slope of the permeance obtained from few days before and after feed sample characterization.

rack 1. Since they contain similar membranes, intuitively it can be expected that rack 1 behaves like racks 2 and 3. The reason for this deviation is not clear, and is most probably due to other factors that were not explicitly measured in this study. Other factors, in addition to feed properties that were analyzed in this study, could have affected filtration performance. Unavailability of those other data for the given racks limits a comprehensive analysis of the filtration performances. As expected, rack 4 showed a different course since the membrane type differed from the other racks. It showed only a weak correlation with the others. Indeed, the behavior of rack 4 could already be clearly distinguished from the others when looking at the permeance profiles (Figure 2.3).

The fouling parameters were also compared with the different feed water

Table 2.3: Summary of the most important feed water parameters and their degree of association with the IFR parameters for all racks. IFR_L: Irreversible fouling rate calculated as the permeance loss; IFR_R: Irreversible fouling rate calculated as rise in filtration resistance.

	IFR_L				IFR_R			
	Rack 1	2	3	4	Rack 1	2	3	4
pTEP		*	*					
cTEP						*		
TEPtot		*				*		
Chla (pond)	**				***	*	*	
Chltot (pond)	**				***	*	*	
D/D' (pond)	**				**			
Pheo (pond)					**			
SS			**				*	
SiO_2								***
Total N			*					
Temperature								(***)
Fe			(*)			(*)		

*** : p<0.01; ** : p<0.02; * : p<0.05

characteristics to be able to point at a possible correlation between them (Table A.3 and A.4 of the appendix). Only feed water characteristics that showed a standard deviation of more than 25% were considered. The feed water characteristics that showed a correlation with the IFRs were TEPs, Chla, Chltot, Pheo, SS, SiO_2, temperature and total nitrogen content. The strongest correlations were found for the chlorophyll values and the IFR_R of rack 1, which means that during algal bloom in the pond, and especially with high Chla concentrations, there seems to be a larger reduction in permeance of the membranes in rack 1. An influence on rack 2 and 3 of these algae parameters was also found, when looking at the IFR_R (taking into account the influence of temperature).

The influence of TEPs was not as large as the influence of the algae parameters, despite a strong correlation between TEPs and the algae parameters (Table A.3). pTEPs gave a weak correlation with the IFR_L for racks 2 and 3, and cTEPs with the IFR_R for rack 2. Of all other parameters, only the suspended solids showed a weak correlation with the IFR_L and IFR_R of rack 3. The suspended solids also gave a correlation with the TEP and algae-parameters (see above), thus a similar influence would be expected. Fe showed a weak inverse correlation with the IFR_L and IFR_R of rack 3. The IFRs of rack 4 did not seem to be influenced by any parameter, except by SiO_2 and temperature.

High SiO_2 seemed to be associated with a steeper rise in resistance to filtration. When IFR_L was considered, this association was not found. The temperature is probably not a causal factor, since it was beginning of winter and the IFR of rack 4 was rising without fall back, in contrast with the other racks (Figure 2.3).

2.3.2 Fouling autopsy

Knowing the origin of the TEPs (from algae mostly in the pond and partly in the canal) and their assumed role on membrane fouling, an autopsy was performed on the two installed sample modules that were taken out just before and just after a CIP to look at the fouling substances of the fouled and cleaned membranes in detail.

Morphological characterization and inorganic fouling

A macroscopic image of the membranes already shows distinct morphological features (Figure 2.4, top row). The fouled membrane shows a marked red color, which is an indication for the presence of significant amounts of Fe oxide. This is a logical consequence of the dead-end configuration of the filtration. Therefore, one can expect that, since iron chloride is dosed in the rack intake, the organic matter (including TEPs) flocculated to form Fe-organic complexes, which are mostly retained on the membrane surface. The loosely attached flocs can be cleaned by backwashing, but parts of them are residual (not removable by CEB, but removable by CIP)/irrecoverable (not removable by CIP) and thus remained on the membrane surface. The residual fouling is the fouling visible under SEM on the fouled membrane, while any fouling visible on the cleaned membrane is irrecoverable fouling (Figure 2.4, bottom row).

Since most of the fouling is irreversible (can be removed by means of CIP), the red color (iron-organic complexes) is completely gone visually on the cleaned membrane, suggesting the effective removal of this compound via CIP. However, the cleaned membrane shows a somewhat yellow color when compared to the white pristine membrane. Also, SEM images of clean and fouled membranes show some clear differences in material on the membrane surfaces in a microscopic level (Figure 2.4). On the fouled membrane, a variety of foulants were clearly seen, which are evenly distributed over the entire membrane surface. Based on the analysis of foulant morphology and recovery after chemical cleanings with oxalic acid, the major foulant accumulated on the membrane seems to be organic matter complexed with Fe oxide. The CIP removed most of these foulants.

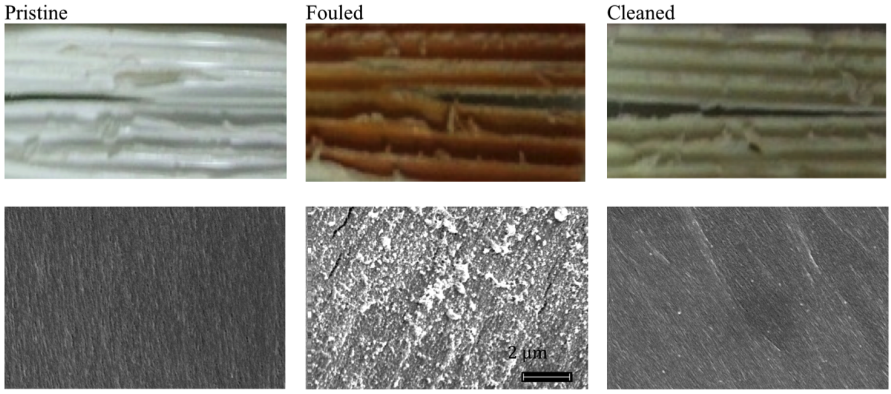

Figure 2.4: Macroscopic (top row) and SEM (bottom row) pictures of the pristine, the fouled and the cleaned membrane.

Organic components in the fouling layer

Analysis of the solubilized foulants with a TOC analyzer showed that the organic foulants consisted of large portions of organic carbon and there was very little inorganic carbon on each of the membranes (0, 27 and 5 mg/cm^2 for pristine, fouled and cleaned membranes respectively). CIP-cleaning removed most of this organic carbon, but not to the level of the pristine membrane. It is very likely that the remaining organic carbon formed recalcitrant complexes with Fe, which are resistant to oxalic acid treatment, thus remained as irrecoverable fouling.

Furthermore, to identify the organic material present on the membrane surfaces of the fouled membrane and the cleaned membrane, ATR-FTIR was used. The ATR-FTIR spectra of the pristine, cleaned and fouled PES membranes clearly show the presence of the chemical bonds originating from the PES material and the foulants (Figure 2.5). The peaks at 3090, 3067, 1650, and 1580 cm^{-1} correspond to the aromatic structures in the PES material [17, 129, 5]. The peaks at 1485, 1240 and 1150 cm^{-1} correspond respectively to S-S stretching, C-C-O vibration and symmetric vibration of the SO_2-group. The peaks at 1040 and 920 cm^{-1} could probably be attributed to preservatives [17], also since they are only present on the pristine membrane. All significant peaks that correspond to the PES membranes and foulants are summarized in Table 2.4.

The presence of polysaccharides and polysaccharide-like foulants could be identified by peaks at 1040, 1100, 1734 and 2880 cm^{-1} that correspond

respectively to the C=O stretching [105] (note that it overlaps with the preservative characteristic), the OH bonds [105], C=O stretching [36, 100] and CH_3 or CH_2 symmetric aliphatic stretches [17].

Other possible foulants are amino acids and humic substances that can be linked to the peak at 1400 cm^{-1} which corresponds to the symmetric stretching of $-COO^-$ [81, 122]. In addition, the protein secondary structure of amide II and I are respectively detected from the two peaks at 1540 cm^{-1} for the N-H bending and the C=O stretching vibration of the protein backbones [9]. The peak at 1650 cm^{-1}, which is stronger for the fouled membrane, could also be indicative for humic substances in the fouling layer [129]. The broad peak between 3700 and 3000 cm^{-1} can be explained in different ways. It could be OH-stretch of bound water, which can point to OH-groups of polysaccharide-like substances [17, 105]. It could also be an amino group [133].

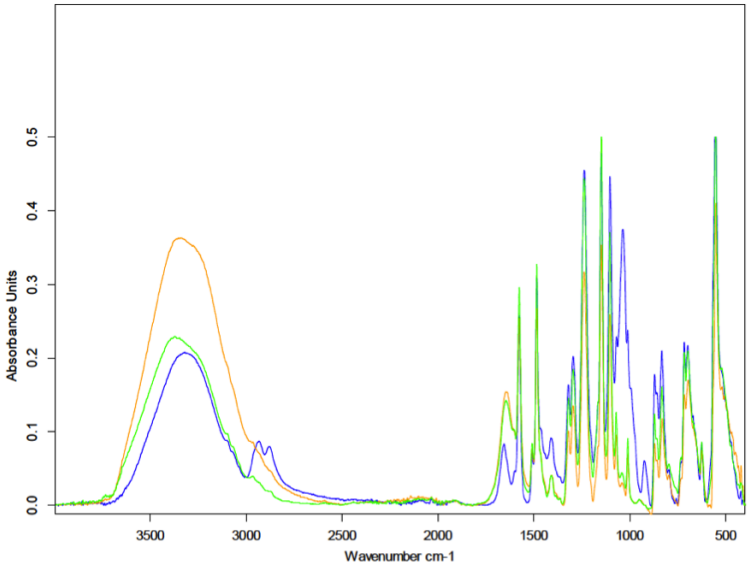

Figure 2.5: FTIR spectrum of the fouled (red), cleaned (green) and pristine (blue) membrane.

When comparing the relative intensities of the peaks among the pristine, fouled and cleaned samples, most peaks overlap with each other and the intensity of the peaks from the pristine sample is higher or similar to the fouled and the cleaned one, except for a peak around 1650 cm^{-1}. This particular peak, indicative for aromatic systems, possibly from humic substances, is present

Table 2.4: The qualitative analysis of different foulants

Wavelength (cm^{-1})	Bond	Substances
2880	CH_3 or CH_2 symmetric aliphatic stretch	Polysaccharides
1734	C=O streching	or
1100	OH-bonds	polysaccharides-like
1040*	C=O-stretching	
1650*	Aromatic system	Humic substances
1640	C=O stretch vibration (backbones)	Amide I for protein secondary structure
1540	N-H bending (Amide I)	Amide II for protein secondary structure
1400	symmetric -COO- stretching	Amino acid and humic substances
3400	OH bound water/N-H stretch	Polysaccharides/protein
3090		
3096	Aromatic CH vibration	
3067	Aromatic CH vibration	
1650*	Aromatic stretching	
1580	Aromatic system	Membrane material (PES)
1485	C-S stretch	
1240	C-C-O-vibration	
1150	symmetric vibration SO_2-group	
2930	CH_3 asymmetrische alifatische stretch/ CH_2 asymmetrische alifatische stretch	Membrane preservatives
2880	CH_3 symmetrische alifatische stretch/ CH_2 symmetric aliphatic stretch	
1040*	C=O-stretch	

* doubles: two possibilities

on the fouled and cleaned samples with similar intensity, suggesting that the organic matter associated with it, is irremovable via the applied CIP.

Inorganic components in the fouling layer

Elemental analysis of the solubilized fouling layer shows that there is a lot of Fe on the fouled membrane, as could be expected (Figure 2.6). The CIP procedure could not remove all Fe, so the irrecoverable fouling layer still contained some Fe, probably as a complex with organics as discussed above. The pristine membrane contained Na (also found on the EDX profiles, see Figure 2.7) and Al, which seemed to be removed after the filtration and the CIP-procedure. No Ca or Mg could be found although they can create more fouling by formation of cation bridges between TEPs or other organic materials [2, 93].

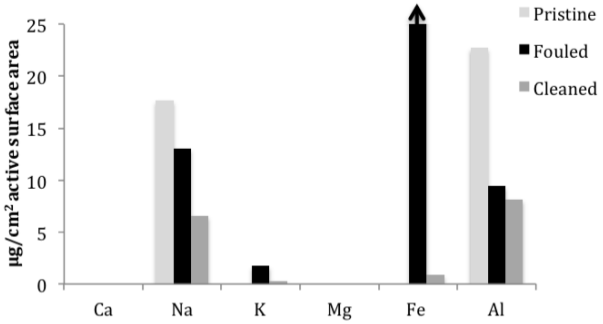

Figure 2.6: The inorganic elements presents in the pristine, fouled and cleaned membrane samples (the arrow indicates that the iron concentration was too high to be measured exactly).

The pristine membrane, in addition to the residues of the coating material (Au and Pt), only shows peaks that correspond to C and O, i.e. no Fe peak. There also seems to be relatively very small peaks corresponding to Na and Cl, possibly from preservatives. The fouled membrane contains a lot of Fe and very little C. The membrane seems to also contain Si, Na, Cl and Ca, but these concentrations are very low and variable (depending on the measurement, see Figure 2.7). After CIP-cleaning the Fe and excess O seem to be largely gone. Only a small portion of Fe (as observed from the EDX spectra) remained on the cleaned sample. This irremovable Fe, most probably present in the form of Fe-organic complexes accumulates and governs the irrecoverable fouling over the

longer term operation. Other metal, such as Co, Al and Cl, were also present but in very small quantities.

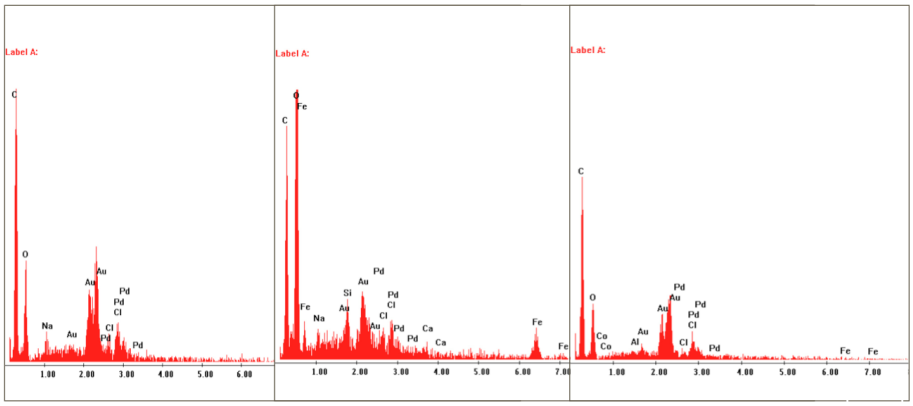

Figure 2.7: EDX spectra of the fouled (left), cleaned (middle) and pristine (right) membrane.

2.4 Conclusions

The link between feed parameters, in particular the TEP content, and the filtration performance of a full-scale UF plant was investigated over an 8 months time period. Many parameters were found to correlate well with IFRs. However, none of the parameters studied is universal, suggesting a more complex mechanism. Overall results suggest that the parameters are highly interrelated. Membrane autopsy suggested the formation of Fe-organic complexes that contributed to the residual membrane fouling. Application of other cleaning agents and other types of flocculant (possibly organic) that can be fully removed, is suggested.

Chapter 3

Role of TEPs in membrane fouling: *Chlorella vulgaris* broth filtration

Adapted from: Discart, V., Bilad, M. R., Vandamme, D., Foubert, I., Muylaert, K. and Vankelecom, I. F. J. Role of transparent exopolymeric particles in membrane fouling: *Chlorella vulgaris* broth filtration. *Bioresource Technology* 129 (2013), 18–25.

Abstract

Recent reports show strong evidence for the involvement of TEPs, mainly produced by microalgae in natural environments, in membrane fouling in a wide range of membrane filtration processes. The objective of this study is to fundamentally investigate the direct role of TEPs on membrane fouling by using different *Chlorella vulgaris* broth solutions and different fractions of such broth (the soluble and bound fractions, the cells separated from these fractions and the cells with their bound sugars, separated from the soluble fraction) as filtration feed. The relation between the feed properties and their filterability over three membranes was determined. Scanning electron microscopy and light microscopy showed that the foulant types differed for each broth fraction and confirmed the role of TEPs in the fouling of microfiltration membranes. In

addition, this study also contributes to the role of TEPs in the filtration of microalgae cultivated for commercial reasons

3.1 Introduction

Recent reports show strong evidence for the involvement of transparent exopolymer particles (TEPs) in membrane fouling in a wide range of membrane filtration processes [41, 151, 79]. Because of the transparent nature of these TEPs, their role in membrane fouling was in the past often overlooked. In addition, they often escaped from standard pretreatments applied prior to membrane filtration because of their gel-like compressibility [79, 152]. TEPs have natural properties of variable size (0.4-200 µm), a gel-like structure and a high negative charge. Early indications of the involvement of TEPs in membrane fouling, possibly by inducing colloidal fouling or biofilm formation, or a combination of both, led to a significant research interest in this area. The influence of TEPs was studied in a wide variety of set-ups, in reverse osmosis [152] and ultrafiltration (UF) [18], as well as in membrane bioreactors [41]. In all these systems, TEPs appear to be involved in the fouling process. In natural environments, TEPs and TEP precursors can originate from human debris, bacteria or multicellular organisms like macroalgae, oysters or sea snails [65, 99], but the majority of the TEP precursors is produced by microalgae in natural environments [113, 50, 15]. Surprisingly, no studies report about the influence of TEPs on the filtration of microalgae cultivated for commercial reasons. In the previous chapter, the influence of TEPs in a full scale natural water filtration plant was investigated, but for microalgae broths, where the TEP concentrations are much higher, the influence on membrane fouling during filtration for harvesting could be much more significant. Microalgae are photosynthetic organisms with an enormous potential for cultivation as energy crops, but there are still major challenges for the large-scale cultivation of these organisms. One of them is the development of an energetically favorable method to harvest the produced biomass [28]. Membrane techniques are an effective method of harvesting microalgae, with advantages such as almost complete retention of biomass, potential disinfection via removal of protozoa and viruses, no or little need to add chemicals to the system and a relatively low energy consumption [28, 85, 23]. The efficiency of the process is however compromised by the fouling of the membrane by microalgae and their residues. Also, because of the difficulty of working with pure microalgae cultures, biofilm formation could become a problem [124]. Furthermore, microalgae are abundant and diverse in drinking water supplies including lakes, reservoirs, rivers, and streams. Occasional algal blooms cause significant challenges in drinking water treatment due to the extracellular release of organic compounds into water, or upon cell lysis. The release rates of these compounds (TEPs and other algogenic organic matter (AOM)) are quite variable depending on the algal growth phase, microalgal species, and their physiological and environmental conditions.

The first objective of this study is to investigate fundamentally the direct role of TEPs on membrane fouling by using five different broth solutions of *Clorella vulgaris* as filtration feed [28]. *C. vulgaris* is a well characterized species of microalgae which is often involved in unfavorable algal blooms [75], but also has an excellent potential for large-scale commercial CO_2 capture and lipid production [91]. The microalgal broth samples were taken from different stages of growth in a batch cultivation culture, i.e. 5 samples were taken over a 21 days cultivation. Factors that are expected to play an important role in membrane fouling were identified and measured, namely the concentration of biomass, soluble microbial products (SMP), extracellular polymeric substances (EPS) (both the proteins, carbohydrates and TEPs), and relevant multivalent cations. The filterability of the 5 feed samples was screened using three different membranes. The relation between sample properties and their membrane filterability was made using the Pearson correlation coefficients. To fully assess the results, a *C. vulgaris* broth solution was additionally fractionated into different solutions containing specific soluble, bound and biomass fractions that were used subsequently as filtration feed to investigate in detail their individual effect on membrane fouling. In addition, scanning electron microscopy (SEM) and light microscopy were also used to analyze membrane samples.

3.2 Materials and methods

3.2.1 Cultivation and determination of microalgae concentration

C. vulgaris (SAG, Germany, 211-11B) was cultured in Wright's cryptophyte medium prepared from pure chemicals dissolved in demineralized water [61]. The algae culture was grown in a plexiglas bubble column photobioreactor, with a working volume of 25 L and diameter of 20 cm. Degassing was carried out with filtered air at a constant flow rate of 4.5 L/min. The composition of the cultivation medium is given in Vandamme et al. [143]. The samples were taken at day 2 (T_2), 4 (T_4), 6 (T_6), 8 (T_8) and after 21 days of continuous cultivation (T_{21}), all stored at 4°C in the dark until being used for analysis or as filtration feed. All samples were fractionated according to the method suggested by Judd et al. [78] to determine the soluble and bound components of every sample (see next section). Hereby, 4 different fractions were obtained per sample:

- The soluble fraction: was obtained as the supernatant of the culture after centrifugation at 4000 g for 5 min.

• The fraction containing the cells with their bound substances (further referred to as 'Cells^{EPS}-fraction'): was obtained by resuspending the pellet, obtained by centrifugation at 4000 g for 5 min, in Ringer's solution (consisting of 0.12 g/L calcium chloride, 0.105 g/L potassium chloride, 0.05 g/L sodium bicarbonate and 2.25 g/L sodium chloride).

• The bound fraction: was extracted from the biomass as the supernatant after resuspending the pellet in Ringer's solution, heating for 10 min at 80°C and centrifuging again at 5000 g for 10 min.

• The fraction containing the cells (further referred to as 'Cells-fraction'): the pellet obtained after the centrifugation-heating-centrifugation procedure was re-suspended again using Ringer's solution to obtain this fraction.

Additionally, a larger quantity of sample T_{21} was fractionated with the same method and the several fractions of T_{21} were used for filtration tests (see Section 3.2.3). Before filtration, the biomass concentration of every sample was determined by several methods. A Coulter counter was used to determine the amount of particles between 2.45 and 10 µm. The dry weight (DW) of the samples was determined gravimetrically by filtration (n=3) using Whatman glass fiber filters (Sigma–Aldrich) and drying until constant weight at 105°C. The optical density was determined at a wavelength of 550 nm, while chlorophyll a (Chla) concentrations were obtained using fluorometry (460 nm excitation, 685 nm emission).

3.2.2 Characterization of microalgae cultures

The microalgae cultures and the different fractions of sample T_{21} were characterized by determining the amount of carbohydrates, proteins and particulate and colloidal TEPs in the bound fraction (referred to as EPS_{CH}, EPS_{PR} bpTEP and bcTEP respectively) and soluble fraction (referred to as SMP_{CH}, SMP_{PR} spTEP and scTEP respectively). The carbohydrate concentration was determined by the phenol-sulfuric acid method [48] and the protein concentrations by the Bio-Rad protein assay. Nitrite and nitrate concentrations were not detected by ion chromatography and thus assumed to be too low to interfere with the obtained carbohydrate concentrations [47]. The concentrations of the multivalent cations Fe and Mg were determined with inductively coupled plasma atomic emission spectroscopy and the calcium concentration with atomic absorption spectroscopy.

The TEP concentrations (both in particulate and colloidal forms) were determined in each fraction by the Alcian Blue (AB) method described by Villacorte et al. [152]. Hereby, between 10 and 50 mL of TEP-containing

solution is filtered over a polycarbonate (PC) filter of 0.4 µm for particulate TEPs (pTEPs) and 0.1 µm for colloidal TEPs (cTEPs). After this, the filter is stained with 1 mL of a pre-filtered solution containing AB and acetic acid at pH 2.5. As also mentioned by Villacorte et al., the calibration procedure is vulnerable to several inaccuracies such as weighing very small quantities of Xanthan Gum (XG) on PC filters and preparing solutions of XG (suspended/colloidal) with uniform properties. Therefore, the calibration procedure was also not applied in this study [152]. However, the AB concentration of the solution was always determined before staining by measuring the absorbance at 787 nm and adjusting it to a value of 0.150 in a multiwell plate reader. This was done for two reasons. Firstly, a staining capacity test showed that the concentration of AB in the dyeing solution has an influence on the measured amount of "TEPs", even when identical samples are used. Secondly, a very strong correlation was found between the copper concentration of an AB solution and the absorbance at 787 nm of the solution ($R^2 = 0.996$), even stronger than with the absorbance at 610 nm ($R^2 = 0.925$), which is supposed to give the highest absorbance of an AB solution in water. An absorption at 787 nm of 0.150 corresponded to an AB concentration of 150 mg XG/L.

3.2.3 Experimental set-up

Membrane properties

Three different membranes were used in this study: two microfiltration membranes: PC 0.4 ($PC_{0.4}$, It4ip) and 0.1 µm ($PC_{0.1}$, Whatman nuclepore) and one polyethersulfone UF membrane having effective pore size of 5 kDa (PES_{5kDa}, Koch). All of them are hydrophilic, having contact angles of 62°, 65°and 69° for $PC_{0.4}$, $PC_{0.1}$ and PES_{5kDa} respectively (all measured by goniometer, FTA2000 Multi-Fluid Programmable Analyzer). The membrane surface microstructures were also observed via scanning electron microscopy (Philips SEM, XL30 FEG, with EDX dx-4i system). The SEM samples were dried and coated with a sputtered gold layer prior to analysis.

Filtration tests

The filtration of feed samples was performed at room temperature in a 200 rpm stirred dead-end filtration cell at constant pressures of 1, 2.5 and 10 bar for $PC_{0.4}$, $PC_{0.1}$ and PES_{5kDa}, respectively. Each pressure was chosen to represent more realistic membrane flux values used in a possible full-scale plant for each corresponding membrane. The feed side was pressurized with

nitrogen gas and the membrane flux was determined by recording the mass of permeate collected over time on a top-loading balance (Mettler-Toledo) using data acquisition software (BalanceLink). Before filtering the samples, the clean water permeance (L_{CW}) of each membrane was determined by filtering demineralized water to obtain 1265, 67 and 11 (L/m^2 h bar) for $PC_{0.4}$, $PC_{0.1}$ and PES_{5kDa}, respectively. This permeance measurement also acted as membrane compaction and conditioning stage prior to the actual microalgal broth filtration.

Characterization of fouling

From the filtration test data, 9 fouling parameters were derived to evaluate the filtration performance, all summarized in Table 3.1. A set of parameters was used since no single universal membrane fouling indicator can cover a wide range of filtration processes accurately.

Table 3.1: Selected fouling parameters.

Symbol	Definition	Unit
$L_{Lost>t15}$	The average rate of permeance loss after 15 min filtration	L/m^2 h^2 bar
$L_{Lost>t25}$	The average rate of permeance loss after 25 min filtration	L/m^2 h^2 bar
L_{t20}	The permeance at filtration time of 20 min	L/m^2 h bar
L_{t40}	The permeance at filtration time of 40 min	L/m^2 h bar
$L_{av>t15}$	Average permeance after 15 min filtration	L/m^2 h bar
$L_{av>t25}$	Average permeance after 25 min filtration	L/m^2 h bar
L_{v10}	Permeance at specific permeate volume of 10 L/m^2	L/m^2 h bar
SFV_{t60}	Specific filtration volume after 60 min	L/m^2
TTF	Time to filter a specific filtration volume of 1 L	$h.m^2/L$

Fouling autopsy

The microstructure of all fouled membranes after the filtration test was observed with SEM. The membranes samples were dehydrated in a series of ethanol/water solutions (25, 50, 75 and 96%), dried at room temperature, and sputtered with a gold layer prior to analysis. The visual observation of membrane samples was also conducted using light microscopy for both $PC_{0.4}$ and $PC_{0.1}$. To determine the presence of TEPs on the fouled membrane surfaces, membranes were stained with AB after filtration of demineralized water (blank) and after filtration of the samples. This way, the TEP fraction of foulants could absorb the colorants and could be seen under a light microscope.

3.2.4 Statistical analysis

The Pearson regression coefficient (r) was used to depict the correlation among samples variables and the correlation between individual sample variables and sample filterability. The Pearson regression coefficient is normally used to express the intensity of a linear relationship between a pair of parameters. Values are between -1 and 1: the closer the values to ± 1, the stronger their relationship. Minimum r values are required to fulfill a certain degree of significance, also as a function of sample number. For the 5 samples used in this study, the degree of correlation between the tested parameters is defined as $0.805<r<0.878$ for low significance (90% confidence level), $0.878<r<0.959$ for medium significance (95% confidence level) and $r>0.959$ for high significance (99% confidence level). They are marked with asterisks *, ** and *** respectively for low, medium, and high significance levels.

3.3 Results and discussion

3.3.1 Membrane and sample characteristics

Membrane

The diverse properties of the three different membranes are described in Section 3.2.3 $PC_{0.4}$ and $PC_{0.1}$ are the membranes commonly used for TEP analysis to determine the colloidal and particulate fractions of TEPs [153, 151, 152]. It is expected that only particulate TEPs would give a significant fouling impact on $PC_{0.4}$, since the colloidal fractions, by definition, should pass through it. The cumulative effect of TEPs would be expected for the membrane with smallest effective pore size (PES_{5kDa}).

Samples

Fresh samples

The feed samples are also very diverse (Table 3.2). This diversity is an important criterium for acquiring the significance of the influence of each variable. TEP concentrations were also relatively high in comparison with natural feeds, such as wastewater effluent, surface water and sea water [151]. The high TEP concentrations are highly desired in this study to better observe their impact on filtration. Indeed, their impact might otherwise be overshadowed by other parameters.

Table 3.2: Composition of the different microalgal based broth feed sample (* : Fractionated from sample T_{21}).

Components	Unit	*Chlorella vulgaris* broth samples								
		Sample T_2	Sample T_4	Sample T_6	Sample T_8	Sample T_{21}	*Soluble fraction	*Bound fraction	*Cells-fraction	*Cells^{EPS}-fraction
Chla	mg/L	8.5	13	16.5	18.1	22.3	0.3	0.2	11.8	17.5
Cell number	10^7 cells/mL	0.7	1.3	2.5	1.7	3.0	0.04	0.03	1.6	2.9
Dry weight	mg/L	140	190	280	230	340	6.5	270	230	400
spTEPs	abs/cm/L	0.7	8.7	7.8	14.6	3.9	3.9	0	3.3	9.7
scTEPs	abs/cm/L	5	15.3	23.7	18	5.7	5.7	0	3.4	5.5
sTEPs (spTEPs+scTEPs)	abs/cm/L	5.7	24	31.5	33	9.6	9.6	0	6.7	15.2
bTEPs (bpTEPs+bcTEPs)	abs/cm/L	12.3	29	24.2	14.1	2.3	0.0	2.3	4.8	19.5
EPS_{CH}	mg/L	3.5	28.6	79.2	47.5	35.6	0.0	35.6	71.2	93.1
EPS_{PR}	mg/L	-	-	-	-	-	-	-	-	-
SMP_{CH}	mg/L	32.4	99.9	71.7	111.9	61.9	61.9	0.00	14.1	60.9
SMP_{PR}	μg/mL	-	-	-	-	-	-	-	-	-
Iron	mg/L	0.9	1.1	0.9	1	0	0	0	0	/
Magnesium	mg/L	1.7	2.1	2	2	0.3	0.3	2.4	2.3	/
Calcium	mg/L	6.2	7.9	7.7	8.7	6.3	6.3	4.4	19.2	/
$Total_{CH}$	mg/L	35.9	128.5	150.9	159.4	97.4	61.9	35.6	85.3	154
$Total_{TEPs}$ (spTEPs+scTEPs)	abs/cm/L	18.0	53.0	55.7	46.7	11.8	9.5	2.3	11.5	34.7

The high TEP-concentrations in these culture samples confirm earlier findings that microalgal EPS is one of the important sources of TEPs in surface water and seawater (Passow, 2001). On the other hand, in the five samples taken as a function of time from the cultivating broth, TEP concentrations did not necessarily increase with algae concentrations. The fifth sample (T_{21}), which contains the highest algal cell concentration and the highest chlorophyll a content, has the lowest carbohydrate concentration (both bound and soluble). Probably, the carbohydrates have been consumed by the algal cells or by bacteria present in the culture. Protein concentrations are too low to be detected by the Bio-Rad assay (detection limit provided by the fabricant is 8 µg/mL). In the paper published by Henderson et al., it was found that protein concentrations in AOM obtained from *C. vulgaris* were about 10 ± 4 µg/mL [66]. Possibly, the growth medium used in this study favors carbohydrate production over protein production, since it contains less nitrogen than the Jaworski's Medium used in their study [66]. Obvious close correlations (between variables that are expected to be closely related) found among variables and their derivatives, e.g. sTEP = spTEPs + scTEPs, are indicated in Table 3.3 by the clusters C1, C2, C3 and C4 (C1: biomass cluster; C2: soluble TEPs cluster; C3: Total CH cluster; C4: Total TEPs cluster). To simplify the analysis, the relations between the variables as individual and/or cluster are studied. Three other important relations among the variables or clusters of variables were observed based on their r-values.

1. In cluster 1, Chla, cell number and dry weight are closely correlated, since all of them represent the amount of biomass in the broths. Apart from that, cell number was found to be better correlated with dry weight than Chla. It can thus be used as a better indirect parameter to represent biomass quantity in microalgal broths. Chla concentrations do not linearly correlate well either with cell density or dry weight, indicating that this pigment is less accurate to represent the biomass quantity. This finding is in line with what is reported by Griffiths et al. [59]: the pigment content of the microalga Chlorella vulgaris can vary between 0.5 and 5.5% of dry weight with age and culture conditions.

2. As for the carbohydrates, the results suggest that TEPs and carbohydrates detected by the phenol-sulfuric acid method do not represent the same fraction, confirming de la Torre et al.[42]. SMP_{CH}, and $Total_{CH}$ are in good association with soluble pTEPs, but not with soluble cTEPs. Because scTEPs constitute a large part of the soluble TEPs (from 51 to 87.7%), the association of SMP_{CH} with sTEPs is lower than with spTEPs. EPS_{CH} is not well associated with the bound TEPs.

Table 3.3: Inter-correlation between samples variables.

	Chla	Cell number	DW	spTEPs	scTEPs	sTEPs	SMP_{CH}	bTEPs	EPS_{CH}	bTEPs/sTEPs	$Total_{CH}$	$Total_{TEPs}$
Chla												
Cell number	** (C1)											
DW	** (C1)	*** (C1)										
spTEPs												
scTEPs												
sTEPs				** (C2)	** (C2)							
SMP_{CH}				**		*						
bTEPs												
EPS_{CH}					*							
bTEPs/ sTEPs	(***)	(*)	(*)									
$Total_{CH}$				**	*	**	* (C3)		* (C3)			
$Total_{TEPs}$					** (C4)	** (C4)		* (C4)				

() indicate inverse correlation, i.e., the r values are negative.
The clusters (C1–C4) indicate clusters of which the parameters correlate because one is derived from the other.

3. All biomass related parameters (C1) correlate well with the ratio bTEPs/sTEPs, but not with bTEPs and sTEPs separately. There seems to be a dynamic behavior of the TEP-concentrations in either fraction corresponding to biomass concentrations and/or growth stages of the microalgae. Closer observation of the values of the coefficient correlation of the biomass-parameters shows that their values are positive for sTEPs, but negative for bTEPs (values not shown in Table 3.3). This means that, in general, as biomass concentration increases, the sTEPs increase but the bTEPs decrease. Closer observation of the values in Table 3.2 show that there seems to be a discrepancy for the samples in early exponential phase and in the later stationary and new exponential phase after continuous feeding of the photobioreactor (see Material and Methods). When growing in batch, during the exponential phase (samples T_2-T_6), the sTEPs seem to increase with biomass, while bTEPs first go up and then down. After the start of the stationary phase (T_6-T_8), the biomass declines and the sTEPs, especially the particulate fraction, increase, while the bound fraction, which is supposed to be assiociated with biomass, decreases. This seems logical since, if the cells lyse, their interiors could form soluble TEPs, as could the TEP-material bound to the cells. After growing in continuous mode with constant feeding (T_8-T_{21}), the biomass has stabilized at higher concentration. The soluble TEPs are lower, possibly consumed by the biomass. The bound TEP-material has also decreased drastically, which means that the cells are less associated with TEP-material. If bTEPs have a function as protective layer for the microalgae cells, this can mean that the cells in sample T_{21} are in a less stressful situation than in the initial samples.

Fractionated samples

After studying the effect of different broth compositions on filtration by filtering the five whole broth samples, the fifth sample (T_{21}) was fractionated and each fraction filtered separately. This was done in an attempt to investigate the individual influence of the different constituents. The fractionation was performed as described in Section 3.2.1. The carbohydrate, protein and the TEP concentrations were determined in the soluble and bound sample fractions, as well as in the Cells-fraction and Cells^{EPS}-fraction (Table 3.2). It is worth noting that only the bTEP concentration as a whole (not particulate and colloidal separately) is considered as an independent variable, since the size of bTEP particles is determined by the way they are detached from the cells during the fractionation process and is of no importance to filtration. Complete cell separation was not achieved by the applied method, given the significant cell amounts that remained in the soluble and bound fraction. In addition, sTEPs and bTEPs were still detected in the Cells-fraction and Cells^{EPS}-fraction. In the Cells^{EPS}-fraction, the TEP-concentrations were even higher than those measured in the whole broth. The cell amount could be minimized (>99%) in the soluble

and bound fraction, but also diminished by 48% in the Cells-fraction. The high amount of TEPs and carbohydrates in the Cells-fraction and $Cells^{EPS}$-fraction can be due to secretion/excretion of those substances after the separation process (centrifugation) by living cells. Alternatively, they might be originating from debris of the broken cells during the separation process. The latter is more probable, considering the high amount of cell loss during the fractionation process. The amount of broken cells can be calculated as the difference between cell density in the whole broth (sample T_{21}) and the sum of the cell densities in the different fractions ($\pm 6.2\ 10^5$ cells/mL after centrifugation and $\pm 1.38\ 10^7$ cells/mL after centrifugation-heating-centrifugation). The presence of cells in the soluble fraction and in the bound fraction shows that the applied fractionation method was not able to separate the cells and soluble fractions completely. This method was suggested by Judd [78] as a standard to fractionate activated sludge samples in SMP and EPS. The poor separation found in this study might be due to the unsuitability of this method for microalgal broth samples. However, no alternative standardized method for microalgae broths exists yet. Because of the aforementioned findings, the effect of some remaining variables associated with different fractions cannot be 100% excluded in each fraction. Therefore, it is expected that some interference with other components might still occur, especially in the Cells-fraction and $Cells^{EPS}$-fraction. It is possible that some fraction of the resistance against filtration by the Cells-fraction is actually caused by the carbohydrate fraction rather than the cells themselves. In the soluble or bound fraction, the presence of cells could still have an influence. This should be kept in mind while examining the filtration results below and in Section 3.3.5 where the foulants are investigated directly through autopsy of the membranes.

3.3.2 Filtration performance

Whole broth samples of T_2, T_4, T_6, T_8 and T_{21} were filtered with the $PC_{0.4}$, $PC_{0.1}$ and PES_{5kDa} membranes. The effects of the membranes and broths themselves on filtration are discussed below.

Effect of membrane

As expected, membranes with a smaller pore size have a lower permeance L. (Figure 3.1). However, their relative permeance (L/L_{CW}) is higher: it is 0.4, 5.2 and 13.9% for $PC_{0.4}$, $PC_{0.1}$ and PES_{5kDa} respectively. Also, higher standard deviations between different membrane samples were found for the membranes with larger nominal pore sizes. This result is common in membrane filtration processes: a steep decline in permeance is expected during the initial stage of

filtration, especially for membranes with large pores due to instant pore blocking by feed particles that have equal or bigger sizes than the membrane pore. Apart from that, back-transport is normally less facilitated in the dead-end system (applied in this study) than in the cross-flow filtration system. The turbulence at the feed-side was also intentionally kept low in this study by applying a rather low mixing speed to avoid changes in the liquid properties, especially in relation to pTEP and cTEPs. For instance, pTEPs could disaggregate due to excessive mixing. High levels of turbulence might also promote detachment of bTEPs.

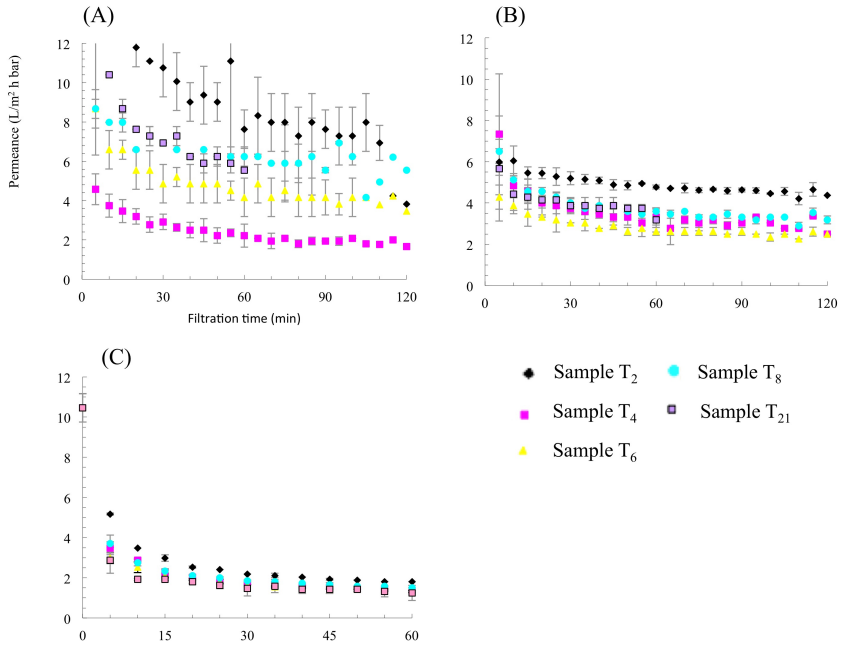

Figure 3.1: Permeance profiles of the microalgae whole broth sample filtrations (A) $PC_{0.4}$, (B) $PC_{0.1}$ and (C) PES_{5kDa}.

Effect of whole broth samples as function of algae culture growth

The effect of sample characteristics on their filterability using different membranes shown in Figure 3.1 is not clear. Sample T_2 shows the highest filtration permeance for all membranes, but the rest of the feed samples showed no such consistent trend. This finding indicates that the effect of certain

sample variables on the filterability might depend on the membrane properties. Therefore, a more comprehensive analysis was performed to link sample variables (Table 3.2) to several fouling parameters (Table 3.1) derived from permeance profiles during sample filtration. To objectively identify these relations, Pearson coefficient correlations were applied, and the results are discussed in Section 3.3.3.

3.3.3 Data analysis: correlations between sample variables and fouling parameters

The summary of the correlation of sample variables with fouling parameters is presented in Table 3.4. This table only shows the variables that are linked with a correlation coefficient of at least 0.81. The complete table with all exact numbers can be seen in Table B.1 of the appendix. The significance levels normally represent how linear the relation is between two coupled parameters. Each pair (sample variable and fouling parameter) that gives the highest level of significance represents respectively the most dominant sample variable to affect fouling from the feed and the most appropriate fouling parameter.

As shown in Table 3.4, no universal sample variable (Table 3.2) or fouling parameter (Table 3.1) could solely explain or represent the filterability of the sample and the permeance of the membrane in all different conditions. The drop in permeance (L_{lost}), specific filtration volume (SFV) and TTF were dominant for $PC_{0.4}$ membranes, while fouling parameters associated with permeance values correlated better with sample characteristics for $PC_{0.1}$. All filtration parameters were suitable for the PES_{5kDa} membrane with rather diverse degrees of significance. Like for the filterability parameter, there was no sample variable that gave a good correlation for all membranes. In an attempt to elucidate the relation between the most significant sample variables listed in Table 3.4 and the 3 different membranes, a Venn diagram of those variables is drawn in Figure 3.2. It is obvious that TEP related parameters dominate for $PC_{0.4}$ and $PC_{0.1}$ (grouped in cluster 2 in Section 3.3.1). This is indisputable, since those two membranes were used to characterize the TEPs. However, no clear distinction between the effect of pTEPs and cTEPs can be observed. By referring strictly to p- and cTEP definitions, one would expect that p- and cTEPs would respectively dominate for the $PC_{0.4}$ and $PC_{0.1}$ membranes. However, the relation shown in Figure 3.2 indicates that all TEP variables contribute significantly with variable levels of significance. It also seems that their size (particulate or colloidal) does not really matter. For the PES_{5kDa} membrane, biomass quantity and bTEP/sTEP variables were dominant. These variables link with TEPs via the latter.

Table 3.4: Coefficient correlation between sample variables and filterability parameters. () indicates inverse correlation.

		Filtration parameters								
		$L_{Lost>t15}$	$L_{Lost>t25}$	L_{t20}	L_{t40}	$L_{av>t15}$	$L_{av>t25}$	L_{v10}	SFV_{t60}	TTF
$PC_{0.4}$	spTEPs	*	*							
	scTEPs	**	**							*
	sTEPs	**	**							*
	EPS_{CH}								(**)	***
	SMP_{CH}	*	*							
	$Total_{CH}$	**	**	(*)					(**)	**
	$Total_{TEPs}$	***	***							
$PC_{0.1}$	scTEPs					(*)	(*)			
	sTEPs					(*)	(*)			
	bTEPs		*							
	EPS_{CH}			(*)	(**)	(*)	(*)	(*)	(**)	**
	$Total_{CH}$				(*)	(*)	(*)	(*)		
	$Total_{TEPs}$					(*)	(*)			
PES_{5kDa}	Chla	**	**	(**)				(*)	(*)	*
	Cell density	**	***	(**)	(*)	(*)	(*)	(*)	(**)	**
	Dry weight	**	***	(**)	(*)	(*)	(*)	(*)	(**)	**
	bTEPs/sTEPs	(*)	(*)	**		*		**	*	(*)

As can be observed in the overview of Figure 3.2, that there is a spectrum of sample variables, ranging from spTEPs from SMP_{CH} that is only dominant for the membrane with the largest pore size, to biomass-related variables that are most dominant for the densest membrane. However, those variables can be linked to each other, indicating they are actually related and there exist some interaction among them. This is very obvious by taking into account the inter-correlation between sample variables discussed in Section 3.3.1. Consequently, any claim on the importance of one particular variable that is solely dominating the filtration performance is not really valid.

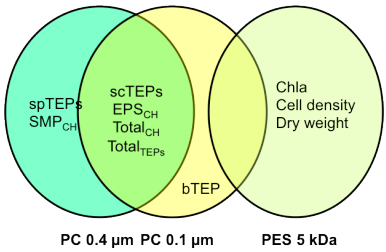

Figure 3.2: Venn diagram showing the relation between sample variables and membranes. bTEPs are not present in Table 3.4, but are included in this diagram, picked as the highest in common "r" for both $PC_{0.1}$ and PES_{5kDa} just below the categorized significance levels.

It is worth noting that the applied statistical analysis method only shows how linear the relation is between coupled parameters. This statistical method does not include simultaneous relations and interactions between different parameters together, which could exist. Their accuracy also depends on the size of the dataset. Only a limited dataset size could be provided in this study due to sample quantity limitation and extensive experimental work required to perform analysis and to produce the data. However, some clear findings could be extracted from the data, as discussed in this paper. Applying other more appropriate statistical methods was not feasible, since most of them require a much more extensive set of data.

3.3.4 Filtration of fractionated samples

Irrespective of the membranes, the trend of the fractionated sample filterability is quite clear, except for the $Cells^{EPS}$-fraction on $PC_{0.4}$ membranes, which has a lower filterability than the whole broth (Figure 3.3). For the other samples, the bound fraction always has the highest filterability followed by both the Cells-fraction and soluble fraction, and then the $Cells^{EPS}$-fraction and

the whole broth, in that order. As discussed earlier, due to the fractionation method limitations, the attempt to completely exclude the variables related to a particular fraction could not be achieved fully. Thus, their individual impact on filterability could not be strictly excluded. Despite this, filtrations show that the soluble fraction, consisting of TEPs and carbohydrates, seems to be of high importance for flux-decline in dead-end filtration in low-pressure microfiltration membranes. This can be seen from the very similar filtration profiles of the soluble fraction and the Cells-fraction, of which the former contains only 2.5% of the total amount of cells, but a much higher amount of soluble carbohydrates. Also the $\text{Cells}^{\text{EPS}}$-fraction, which contains 97% of the original cell amount, but a lot more TEPs and carbohydrates (probably due to breakage of the lost cells), has a lower permeance than the other fractions, in one case even lower than the whole broth. For the UF membrane, presumably the high pressure pushes all cells in a dense cake layer at the feed side which will determine the permeance, even if only few cells are present.

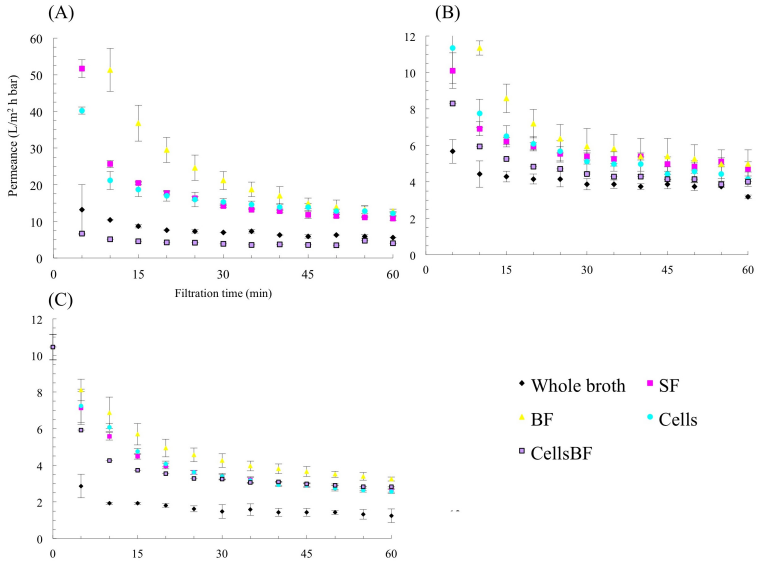

Figure 3.3: Membrane permeance profile during the filtrations of the fractionated samples using (A) $PC_{0.4}$, (B) $PC_{0.1}$ and (C) PES_{5kDa}.

Although there is some clear trend, the permeance values of different fractions are rather close to each other. For a more comprehensive study, the number of feed samples should be increased and the dataset should be analyzed using alternative statistical methods. The effect of an individual sample variable

might then be identified, including its relations with other sample variables. Alternatively, a model sample could be used by using single components or variables, and later followed by samples containing multi-component variables. This systematic study could lead to a more systematic understanding about the effect of an individual sample variable and its interactions.

3.3.5 Fouling autopsy

Scanning electron microscopy

SEM images of clean and fouled membranes show some clear differences in fouling material on the membrane surfaces depending on the feed used (the images can be seen in Figure 3.4). In general, there seem to be many microalgal cells (usually large round white structures on the images) attached to the surface, except for soluble fraction and bound fraction-fouled $PC_{0.4}$ and $PC_{0.1}$ fouled with soluble and bound fraction. Yet, even on these membranes, some cells are still visible which are present due to the incomplete separation obtained via the applied fractionation method (see Section 3.3.1). Some other rod shaped cells are also visible, probably bacteria that could grow in balance with the microalgal cells in the non-axenic culture. Individual TEP particles do not seem to be visible on the membrane surfaces, although some matrix-like structures seem to be surrounding the cells on the $PC_{0.1}$ membranes fouled with Sample T_{21} and the $Cells^{EPS}$-fraction. Fouled PES_{5kDa} membranes always seem to be covered with a lot of cells (algae and other). So possibly, the loss in permeance of this membrane could be attributed to the cells. In conclusion, although from filtration data it seems that the soluble and bound fractions contain fouling material, this is not visible on the SEM images, except for the PES UF membrane. For that membrane, the cells seem to be the fouling material, even though they are present in a much lower concentration.

Light microscopy

The presence of TEPs on a fouled microfiltration membrane on top of the membrane surface can clearly be seen from the blue color after staining the membrane with AB (microscopic pictures can be found in Figure 3.5). Their presence is hardly visible in the non-stained sample making them often overlooked. The blue color spreads evenly over the membrane surfaces, but some more intense blue clusters are visible. This is most probably a reflection of TEP clusters that block the membrane pore-mouth. Several somewhat bigger clusters are also visible, which most probably come from particulate TEPs.

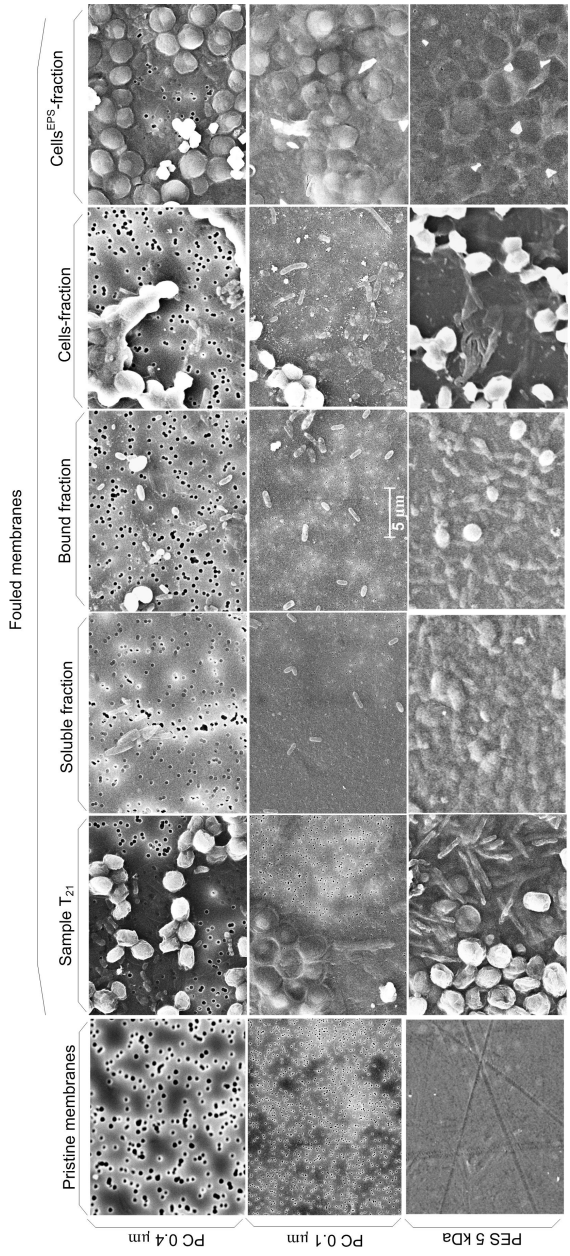

Figure 3.4: SEM images of top surfaces of the pristine membranes and the membrane samples fouled after filtering 5 different fractions.

The microscopic pictures suggest that both p- and cTEPs might play a role in fouling the membranes. Due to their sticky properties, TEPs of any sizes will stick on the surface of the membrane. They accumulate and eventually form clusters that are big enough to block the pores as can be seen in Figure 3.5 D.

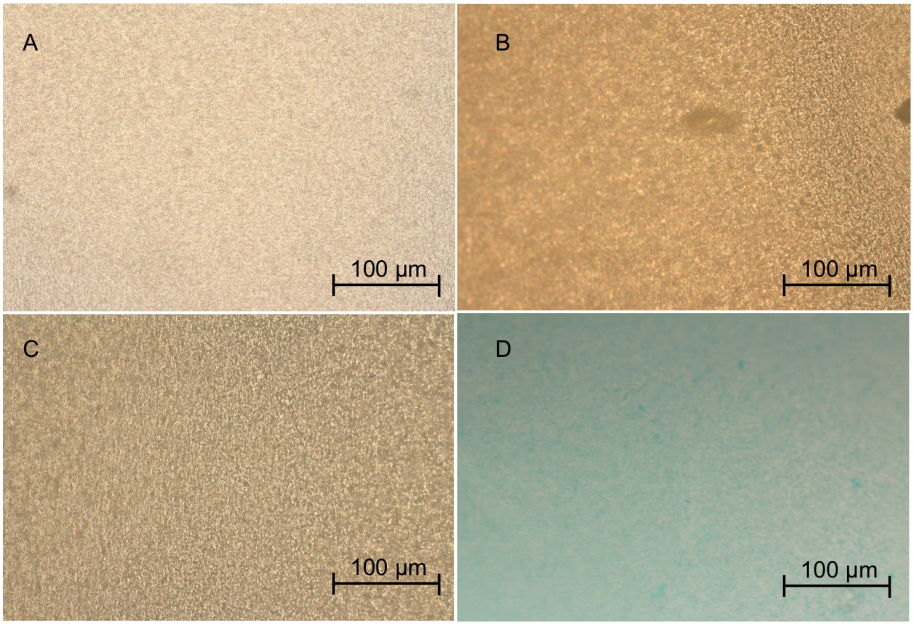

Figure 3.5: Light microscopy pictures of the $PC_{0.4}$ membrane: (A) pristine, (B) after filtration of the soluble fraction of the algae culture, (C) fresh membrane after staining with AB, and (D) after filtration of the soluble fraction and subsequent staining with AB.

3.4 Conclusions

Membrane fouling by microalgae and their residues largely influences the efficiency of membrane filtration processes, as applied for microalgal biomass harvesting or purification of waters contaminated by microalgae. The statistical analysis discloses that no universal sample variable and fouling parameter could solely explain or represent the filtration performance. However, soluble compounds, TEPs and carbohydrates, seem of high importance for flux-decline in dead-end filtration, using low-pressure microfiltration membranes. This is

inconclusive for UF membranes, where the higher pressures presumably push all cells in a dense cake layer that determines the permeance.

Chapter 4

Impact of changes in broth composition on *Chlorella vulgaris* cultivation in a membrane photobioreactor (MPBR) with permeate recycle

Adapted from: Discart, V., Bilad, M. R., Marbelia, L., and Vankelecom, I. F. J. Impact of changes in broth composition on *Chlorella vulgaris* cultivation in a membrane photobioreactor (MPBR) with permeate recycle. *Bioresource Technology* 152 (2013), 321–328.

Abstract

A membrane photobioreactor (MPBR) is a proven and very useful concept in which microalgae are simultaneously cultivated and pre-harvested. However, certain parameters, such as the accumulation of algogenic organic matter, including TEPs, counter ions and unassimilated nutrients due to the recycling of the medium is still unclear, even though the understanding of this behavior is

essential for the optimization of microalgae processing. Therefore, the dynamics of these compounds, especially TEPs, during coupled cultivation and harvesting of *Chlorella vulgaris* in an MPBR with permeate recycle, are addressed in this study. Results show that TEPs are secreted during the algae cell growth, and that their presence is thus inevitable. In the system with permeate recycle, substances such as counter ions and unassimilated nutrients accumulate in the cultivation reactor. Algae growth limitation seems to be linked with these factors, but also with the occurrence of bioflocculation.

4.1 Introduction

Microalgae have been the subject of research for decades, especially since the 1980's, due to their possible use for biodiesel production. Despite their high production costs and due to the rising prices of conventional fuels and the global warming problems, microalgae keep reappearing as a more promising feedstock option than other bio-based crops [58]. Nevertheless, it seems very unlikely that the process will be developed with biodiesel as the only end-product [154, 84]. Microalgae can be of interest for other industries, e.g., as raw material for high-value products [34], or for the treatment of wastewater [110]. Still, for long-term sustainability, all processing stages of microalgae should be simplified and energy input should be substantially decreased [84]. The cultivation and the dewatering stage are two of the most critical stages where improvement is needed [58].

Open raceway ponds and closed photobioreactors (PBRs) are two common cultivating strategies for microalgae [58]. Closed PBRs, despite being more expensive in operation, offer several advantages over raceway ponds, such as limited contamination, higher culture densities and better control over physico-chemical conditions. The biggest limitation on productivity in PBRs is the inherent biomass wash-out, which is the disappearance of the microalgae due to a too high dilution rate (too short residence time), resulting in a harvesting rate (via the outlet) that is higher than the reproduction rate (growth). To prevent this, decoupling of the microalgal biomass retention time (MRT) and the dilution rate (D) is needed, for instance by operating the PBR in membrane photobioreactor (MPBR) mode by coupling the cultivation tank to a membrane filtration unit. The membrane provides complete retention of microalgal cells, thus preventing wash-out and increasing the maximal biomass concentration in the bioreactor, while the medium (water and remaining nutrients) passes as permeate. The biomass concentration can also be better controlled with a separated filtration tank by partly returning the retentate to the MPBR. Recently, the effectiveness of the MPBR system for microalgal biomass cultivation and pre-harvesting was proven [26, 69]. Because of the higher flexibility and robustness, the MPBR could operate at higher dilution and at higher growth rates, resulting in a 9× higher biomass productivity compared to the PBR [26]. In addition, pre–harvesting could be achieved by applying a high concentration factor. The remaining nutrients in the permeate could be recycled to the reactor as feed medium with minimum effect on the growth. This way, a substantial reduction in the water footprint and in nutrient costs is achieved [26]. Recycling culture media is even considered a key issue for the development of large-scale cultures to minimize water and nutrients consumption [62, 63], especially considering the depleting sources of important

nutrients, e.g., phosphorous. Another significant advantage of MPBRs is that they can serve as an effective way of combining wastewater treatment with biomass production [69]. Although the MPBR shows many advantages, close monitoring is necessary, especially when permeate recycle is used. Accumulation of substances such as metabolite products, algogenic organic matter (AOM, organic materials produced by microalgae), counter ions and non-assimilated nutrients are expected to accumulate, which may hinder the prospect of MPBR technology. Understanding and remediating those detrimental effects is key to the good functioning of MPBRs for simultaneous cultivation and pre-harvesting of microalgae.

In the previous chapter, the role of TEPs in the dewatering stage of microalgae cultivation was addressed, but TEPs could also be of importance in other steps of the process. In this study, TEPs were investigated as part of a larger monitoring study, to look at the possible influence on productivity in an MPBR system: for this *C. vulgaris* was grown for 75 days in a lab-scale MPBR system with permeate recycle in batch and in continuous operation. The latter was the continuation of a previous study [26], now operated at different dilution rates. The batch cultivation was performed to observe the behavior of TEPs and other parameters in absence of any dilution. In the continuous cultivation, the system performance was continuously monitored with main emphasis on nutrients and accumulation of AOM, represented in this study by total organic carbon (TOC) and TEPs (a relatively new parameter in algal research), in particular to their impact on growth. In addition, the influence of the permeate recycle (containing accumulated non-limiting nutrients) was also addressed.

4.2 Materials and methods

4.2.1 Microalgae species, growth medium and analysis

C. vulgaris (SAG, Germany, 211-11B) was cultivated in Wright's cryptophytes (WC) medium, prepared from pure chemicals dissolved in demineralized water. The substrate stock solutions were prepared at high concentrations and stored in the dark at 4°C. *C. vulgaris* is a well-characterized microalgae species that has an excellent potential for CO_2 capture and has a considerably high lipid content. It is one of the few microalgal strains that is considered suitable to be cultivated at large scale [91]. 40 mL of sample was taken from the feed, bioreactor, retentate and permeate for further analysis on a daily basis by temporarily opening the bioreactor lid (Figure 4.1, and stored in the dark at 4°C until analysis.

Biomass: dry weight and microscope observations

The biomass concentrations of the samples were determined twice a week by measuring the dry weight of the samples after filtration (n = 2) using Whatman glass fiber filters (Sigma–Aldrich) and drying until constant weight at 105°C. In addition, the optical density was determined at a wavelength of 550 nm. Microscope observations were done to monitor the biomass and make sure that contaminating species were not taking over the broth solution, since the algae were grown as a non-axenic culture.

Conductivity and total organic/inorganic carbon

The conductivity of the feed, retentate and permeate was measured twice immediatly after sampling using a conductivity meter. The conductivity measurements were done to evaluate the ion accumulation as a result of permeate reuse from the membrane filtration as medium in the MPBR. When enough samples were gathered, the organic and inorganic carbon was measured using a TOC analyzer (Multi N-C 2100). In this case, organic carbon can be used to indirectly represent the abundance of AOM in the feed, broth, product and permeate.

TEP concentrations

TEP concentrations in duplicate (with a third measurement in the case of a large discrepancy between two values) were determined twice a week according to the method developed by Arruda Fatibello et al. [6], at pH 4 and at pH 2.5 [45]. The measurement at pH 2.5 was performed to enable the comparison with TEP obtained by other methods, since Alcian Blue (AB) specifically stains certain compounds at pH 2.5. Usually, staining with AB is done at pH 1 or 2.5, depending on the material targeted [80, 115]. In short, 2 mL of sample is stained with 0.5 mL of a 0.06% AB solution after addition of a 0.2 mol/L acetate buffer solution or glycine–HCl buffer until a final volume of 10 mL (for pH 4 and pH 2.5, respectively). Afterward, the mixture is stirred for 1 min and centrifuged at 3000 rpm (2160 g) for 30 min. The absorbance of the supernatant (excess AB solution) is measured at 602 nm to determine the amount of AB that has formed the complex with TEPs. The absorbance is measured at 602 nm, since this is the maximum absorbance of AB in water, as opposed to AB in sulfuric acid, where the maximum absorbance is at 787 nm.

4.2.2 Experimental set-ups and system operation

PBR-Batch

The experimental set-up of the PBR and MPBR is shown in Figure 4.1 A. First, the 25 L cylindrical PBR (part of the MPBR set-up) was operated batch-wise for one week until the microalgae growth reached the stationary phase. Samples were taken twice a day and analyzed for microalgal biomass, TEP and TOC-concentrations. The batch-wise cultivation in the reactor of the MPBR was done twice; once before the MPBR operation and once again after the MPBR operation. The continuous cultivation in the MPBR system was done for 50 days and the second fed-batch cultivation 15 days.

MPBR

The MPBR was operated as the continuation of an earlier study [26], at different dilution rates (D = 0.20-0.36 d^{-1}) for 58 days. Except for the applied dilution rates, the whole set-up, operational conditions and membranes were similar to the earlier study. Briefly, in the MPBR, the culture broth was circulated into a 4 L filtration tank where it was split into retentate (product) and permeate streams with a volumetric concentration factor (ratio of feed and retentate) of 4.4×. The retentate became the pre-concentrated/harvested product and the permeate was collected and recycled as feed medium after addition of the required concentrated stock substrates. The volumetric mass balance of the systems is presented in Figure 4.1 B. Pressurized air sources supplied CO_2 into the culture medium (after being filtered) at a fixed flow rate (5 L/min). The PBR and MPBR were operated under constant light (with no dark phase) without temperature or pH control. To maintain high biomass concentrations in the bioreactor and to prevent wash-out, the broth in the filtration tank was also partly recycled into the reactor. Some fresh demineralized water was introduced to make up the volume that was harvested as retentate or that disappeared through evaporation (±1 L d^{-1}). Since no membrane replacement was done, the filtration fluxes changed when different dilution rates were applied. However, the performance of membrane filtration is not a focus of this study.

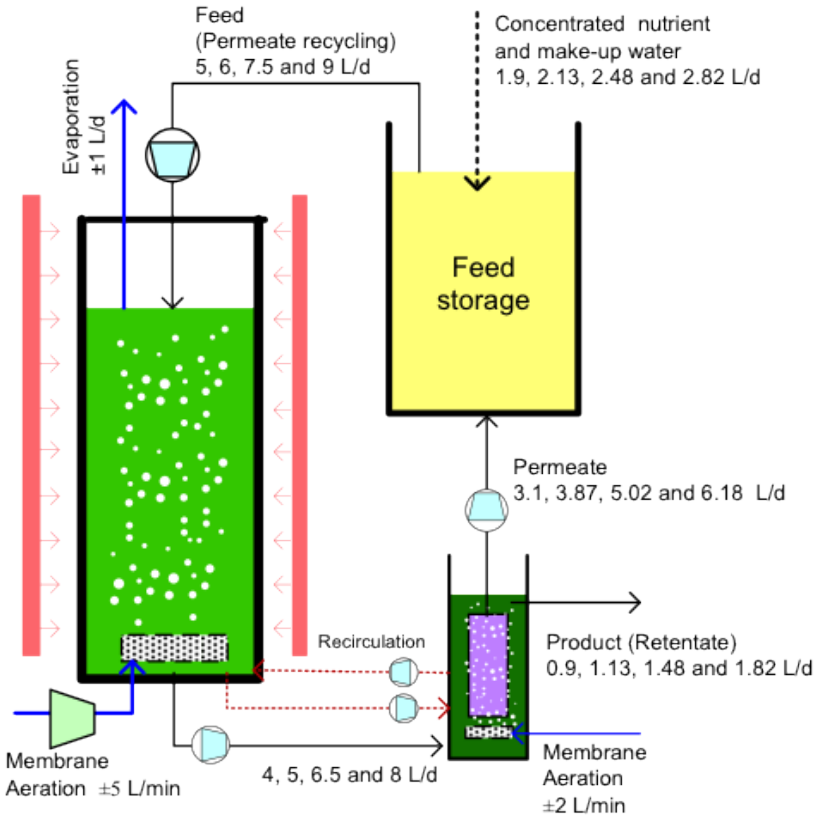

Figure 4.1: Schematic illustration the PBR (A) and MPBR (B) set-up showing the water balances of different dilution rates (0.2, 0.24, 0.3 and 0.36 d^{-1}). The bioreactor was exposed to a constant air supply rate (5 L d^{-1}) from a pressurized air source and to light (2 x 36 W, Sylvania, Germany). A 40 mL sample was taken daily from the PBR, permeate and retentate.

4.3 Results and discussion

4.3.1 Considerations about MPBRs with permeate recycle

Although the MPBR shows many advantages (see Introduction), close monitoring is necessary, especially when permeate recycle is used. Accumulation of substances, such as metabolite products, algogenic organic matter (AOM, organic materials produced by microalgae), counter ions and non-assimilated

nutrients is expected, which may influence various aspects of the MPBR (see below). Understanding and remediating the possible detrimental effects is key to the good functioning of MPBRs for simultaneous cultivation and pre-harvesting of microalgae. Medium recycle and feeding the bioreactor with a constant nutrient composition (WC medium) would at least cause four effects:

(1) The accumulation of non-limiting nutrients, because they are only partly assimilated. This changes the overall feed medium composition and most probably the cell contents [106] and can limit the growth at high concentration factors [4, 88, 126, 63].

(2) Not only the non-limiting substrates and the organics, but also the counter ions of nutrients that are not or poorly assimilated by microalgae, will accumulate in the supernatant (i.e. in the WC medium used in this study, sodium and potassium act as counter ions for NO^{3-} and PO_4^{3-}, respectively), leading to a change in medium salinity which may negatively affect the biomass growth [4].

(3) AOM and TEP concentrations are expected to increase constantly, especially since an elevated biomass concentration and enhanced growth rates are expected. Also TEPs are partly retained by the membrane, so organic metabolites would certainly accumulate, which can lead to a decrease in biomass productivity [126, 30]. AOM has been extensively studied [66]. It mainly consists of polysaccharides (80-90%) that form dynamic micro-gels, and are known as the main constituents of TEPs. In comparison to the Dubois assay, commonly used as a representative test for AOM, a different carbohydrate fraction is measured by the AB method for TEP measurement. The TEP staining method has several advantages over the Dubois method: the dye is non-toxic and no strong acids are used, so that there are no hazardous residues after the test. No special correction is needed for the presence of nitrate and nitrite, which is necessary for the Dubois assay [47]. AOM and TEPs are very important in microalgae production because of four main reasons: (a) they could reduce the potential biomass yield from the assimilable inorganic carbon; (b) they can become an organic carbon source that allows growth of bacteria, which would also consume the nutrients; (c) they can increase coagulant/flocculant loading due to their high negative charge during the harvesting process; (d) they promote membrane fouling together with the microorganisms present in the broth when membrane filtration is used for harvesting. Especially TEPs have been assumed to have a large impact on membrane fouling and water quality parameters (in the case of water purification), possibly even more than the microalgae cells themselves [66, 67, 148, 46]; (e) some organic metabolites that are naturally excreted by microalgae during growth or which are suddenly released when cell lysis occurs, can have toxic effects, e.g. fatty acids and substances derived by oxidation [158, 30, 159]. These toxic effects have especially been noticed when working with high cell concentrations [77, 123]. However, *Chlorella vulgaris* has been

found to not secrete growth inhibitors at high cell densities [92]. During cultivation in a standard growth medium with various dilution, a low sensitivity to growth inhibiting substances was observed [29]. The excretion of AOM, both the amount and the type, is dependent on several properties of the microalgal broth, such as broth age, microalgae species, biomass concentration, and the occurrence of stress factors (such as an increase in salinity) [66]. The dynamics of AOM and TEPs in this study are presented in Section 4.3.4.

(4) There is a possibility of bioflocculation because of higher growth rate, high pH, increasing counter ion concentrations and ageing.

When monitoring for this study started, the MPBR had already been running for 58 days of continuous cultivation [26]. During that period of 58 days, the medium was recycled more than 13 times, but there had been no indication of growth limitation by non-limiting substrate accumulation, nor from metabolite inhibition. However, even though no growth limitation was observed, the composition of some monitored non-limiting substrates was very high, often higher than the composition in the feed. For instance, the total nitrogen in the system (bioreactor, permeate and retentate) was $\pm 4\times$ higher than the one in the fresh feed (prepared using demineralized water) (results not shown). The accumulation of salts was also evident in the previous study, considering the increase in conductivity at a similar magnitude (fresh feed 129 $\pm$ 37; MPBR 471 $\pm$ 49 µS) [26]. It is obvious that the steady-state concentrations of the non-limiting substrates were too low to substantially inhibit the growth in the previous study. The growth limitation found in this study (Section 4.3.3) is probably due to other factors or a combination of many factors, which are discussed in detail in the next sections.

4.3.2 Batch cultivation

During batch operation, the biomass seemed to grow at a linear rate (Figure 4.2 A and B), without clear evidence of an exponential phase, until it reached the stationary phase. The TEP (pH 2.5) concentrations increased almost proportionally with the increase of biomass concentration, at a ratio of 18.68 mg Xanthan Gum (XG) equivalents/g biomass (run 2). Pearson correlation coefficients (r) of 0.825 and 0.816 were found for batches 1 and 2, respectively, which gives a p value below 0.01 for both. This is strong evidence that the microalgal cells produce TEPs during growth, as found in other studies [63, 66, 72]. It is worth noting that even though TEP concentrations were almost proportional to the biomass concentrations, their values undergo somewhat more drastic changes and TEP measurements have rather large standard deviations

(1-338%). This is most probably due to the limitations of the TEP measurement method and the dynamic nature of TEPs [45].

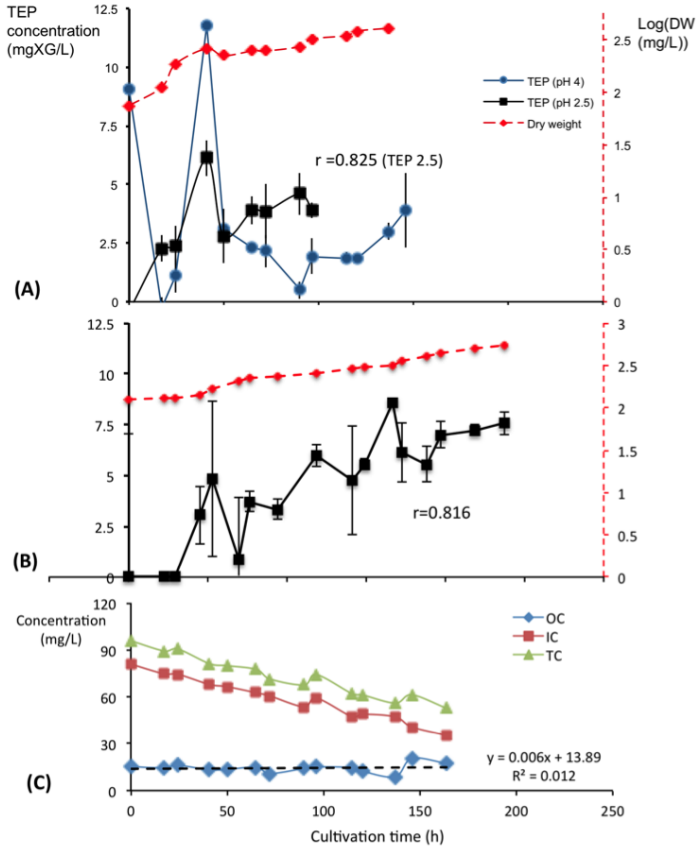

Figure 4.2: The dynamics during batch cultivation of the biomass and TEP concentrations ((A), first batch) and ((B), second batch); and the profile of the carbon concentrations ((C), second batch). OC = organic dissolved carbon, IC =, dissolved inorganic carbon (IC) and TC = total dissolved carbon. The "r" is Pearson correlation coefficient between biomass and TEPs (pH 2.5).

4.3.3 MPBR with permeate recycle: biomass profile

Figure 4.3 shows the biomass profile during continuous cultivation in the MPBR system for 50 days followed by 15 days of fed-batch cultivation. The obtained biomass concentration during the continuous cultivation is significantly lower than in the previous study [26], indicating lower growth rates.

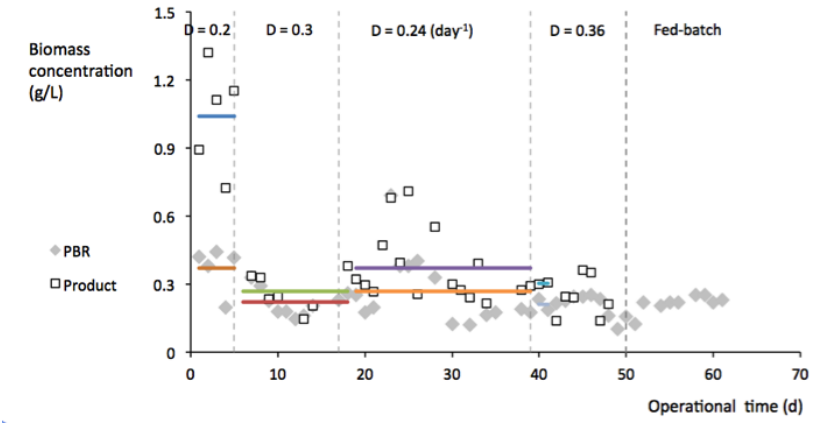

Figure 4.3: The profile of the biomass concentrations in the MPBR system. The concentration of product/permeate is higher than in the PBR because of the pre-harvesting via submerged membrane filtration. Six straight lines represent the average value of biomass concentration from day 1 to 5; day 5-18 and day 18-39 (for each interval, the upper and lower line represent the product and PBR broth respectively). D: dilution rate (d^{-1})

Bioflocculation of microalgal biomass was observed since the previous study. This effect can be due to medium recycle which increases the concentration of counter ions (including multi-valent cations, such as Ca^{2+} and Mg^{2+}), which in combination with a high pH (>8.5, 8.5-9 as observed during this study) promotes bioflocculation [130, 140]. Bioflocculation lowers the number of free cells and the concentration of biomass in the bulk, which in turn lowers the growth rate and volumetric productivity of the system. Thus, although it can be a wanted phenomenon for facilitated harvesting in other systems, it has detrimental effects on the MPBR system.

4.3.4 Dissolved carbon and TEPs

Dissolved carbon

Figure 4.2 C shows the behavior of organic dissolved carbon (OC), dissolved inorganic carbon (IC), present in forms of CO_2, HCO_3^- and CO_3^{2-}, and total dissolved carbon (TC) in the *C. vulgaris* broth during the second batch cultivation. The IC continuously decreased over the cultivation time because of the continuous assimilation into microalgal biomass. This was also represented by the increasing pH of the broth, since no pH control was installed. On the other hand, OC represents the abundance of organic carbon containing substances, including algogenic organic matters (AOMs). Only a small increase of OC was observed as a low but positive slope of the trend line. This result is rather surprising, since AOMs were constantly produced during microalgal growth (see below).

The concentrations of OC and IC in the permeate, in the PBR broth and in the retentate were also measured during the cultivation in the continuous MPBR to monitor the behavior of AOMs (Figure 4.4). High IC values confirm that the system was not under inorganic carbon limitation. OC-results showed that there is no significant difference between the PBR, the retentate and the permeate, meaning that the OC was not effectively retained by the membrane. Their low value is rather surprising when considering some visual evidence of the OC accumulation. Indeed, some physical changes were observed during the operation: foaming at the surface of culture medium and changing of the broth color from dark green to yellowish green toward the end of operation. The former effect suggests the excretion and the accumulation in the supernatant of surfactants such as proteins, amino acids, lipids, and polysaccharides as a result of the death or decomposition of cells. The latter suggests the ageing and (in general) environmental stress (light, temperature, pH, salinity or the presence of other microorganisms) [123, 63]. It is likely that the OC was bound to the cell wall and settled together with the cells during sample storage before analysis: for the OC analysis, the samples were stored in closed glass containers at 4°C in the dark until enough samples were obtained (they had to be analyzed simultaneously).

TEPs

The TEPs were measured at two pH values (2.5 and 4), and their profile is presented in Figure 4.5. An AB solution at pH 2.5 (without extra electrolyte) stains both carboxylated and sulfated polysaccharides instantaneously, but not neutral sugars. At pH 1, it specifically stains sulfated polysaccharides, while

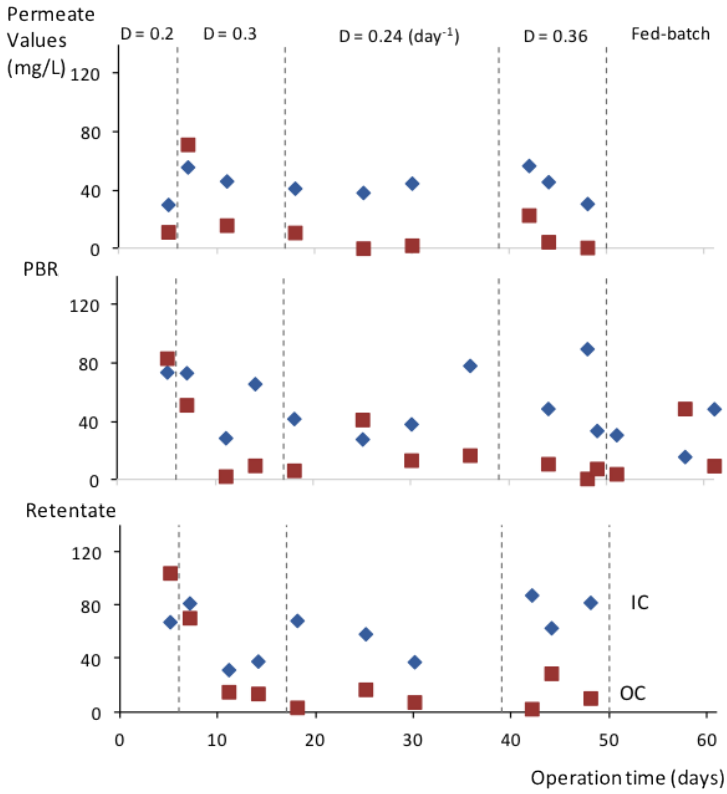

Figure 4.4: Profile of organic and inorganic carbon in the permeate, PBR and retentate.

polysaccharides with carboxyl groups are not stained at such low pH [115]. It is still unclear which substrates AB stains at pH 4.

When comparing the profile TEP obtained at pH 2.5 and 4 during the first batch cultivation (Figure 4.2 A), it seems that AB stains a broader range of substrates at pH 4. A high TEP value at first sampling indicates that some substrates from the feed (WC medium) were stained by AB at pH 4, but not at pH 2.5. They are most probably from vitamin solutions and/or some organics from the inoculum. However, the TEP values dropped in the next few time points, suggesting that they were consumed over time. It implies that in addition to the carboxylated and sulphated polysaccharides stained at pH 2.5, AB also stains carboxyl groups associated with proteins at pH 4 and (at elevated temperatures

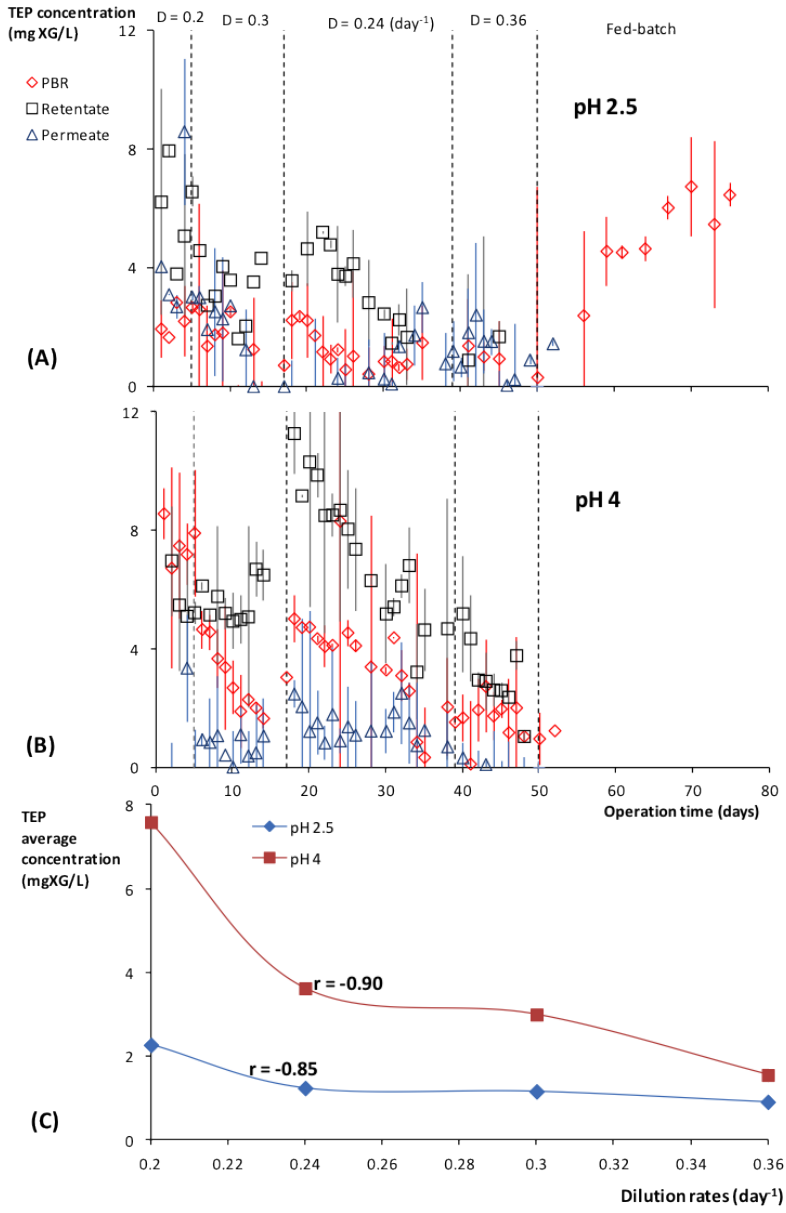

Figure 4.5: TEP profile at pH 2.5 (A) and 4 (B) of PBR retentate and permeate over the cultivation period and (C) the relation between average TEP concentrations in the PBR and the applied dilution rates. "r" is the Pearson correlation coefficient between TEPs measured at two pHs.

or after prolonged staining) also nucleic acids [64]. This means that more substrates are stained at pH 4, which results in higher TEP concentrations, as also shown in Figure 4.5 B (trend line slope of 1.24). When both TEP concentrations were compared with a Pearson correlation, a positive correlation coefficient (r=0.58) was found, which indicates that measurements at both pH have positive association. This means that when TEP-values at pH 2.5 increase, the TEP-values at pH 4 also increase. It is worth noting that many TEP-values on the permeate were below the detection limit. The applied membrane seemed to retain the TEPs only partly, as the TEP concentrations in the retentate were higher than in the PBR, followed by the permeate (which has many samples with TEP-concentrations under the detection limit) (Figure 4.5). Consequently, TEP accumulation was expected. However, no severe accumulation was observed during the continuous cultivation period. Even at steady state, the concentration never exceeded 12 mg XG/L. The TEP concentration is certainly a function of dilution rates, increasing at lower dilution rate (Figure 4.5 C), somewhat like the profile of biomass. However, judging from the low OC value, it is very likely that settling of some macro TEPs muffled the accumulation, as might permeation of dissolved TEP and consumption by some bacteria present in the broth. This was also indirectly observed elsewhere [63]. Nevertheless, the TEP accumulation can be clearly observed the last 25 days when the system was operated in fed-batch mode.

4.3.5 Salt accumulation

The accumulation of salts during the cultivation is reflected by the increase in conductivity (Figure 4.6). As expected, no difference was found between the three measured streams (product, PBR and permeate), because the microfiltration membrane cannot retain the ions. Operating the system in fed-batch mode also resulted in elevated conductivity. Results logically show that the conductivity decreases with increasing dilution rates. When the D changed from 0.2 to 0.3 d^{-1}, salts decreased, followed by an increase when D was turned to 0.24 d^{-1} and finally decreased again after the D changed to 0.36 d^{-1}. One could imagine that at high D, more fresh feed (which has a lower conductivity than the solution in the PBR) is introduced to the system, which induces further dilution.

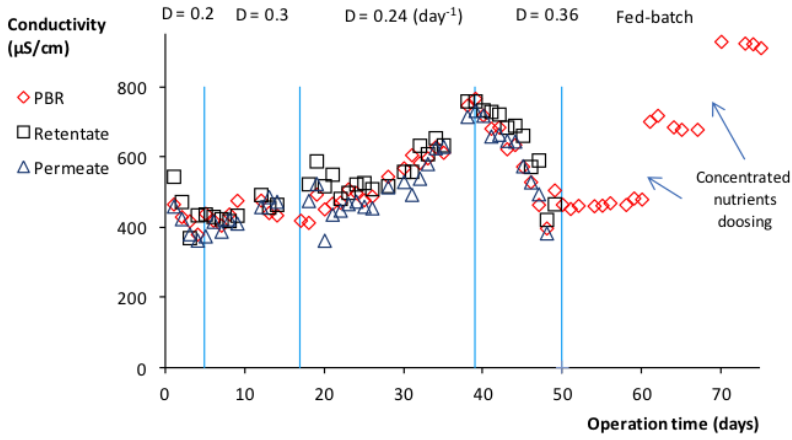

Figure 4.6: The conductivity profile of the retentate, the broth in the PBR and the permeate.

The fact that the obtained conductivity is higher than the estimated value suggests that water evaporation plays a crucial role. Theoretically, the steady state conductivity should be equal to fresh feed multiplied by the concentration factor. Every day, due to water evaporation, one liter of medium was manually added to the PBR to keep the working volume constant. This means that over the MPBR operation ±50 L of water (50 days) was made-up, corresponding to twice the PBR working volume. This evaporation inevitably contributed to an increased conductivity. After certain reuse, the level of conductivity might endanger the growth. *Chlorella* sp. was shown to be unable to adapt to concentrations of more than 1 M NaCl, while growth was inhibited at concentrations higher than 0.2 M as compared to a control in freshwater [4]. A solution having a conductivity of 1 μS/cm is an equivalent of about 0.6 mg NaCl per kg water. Proper formulation of growth medium allowing maximum nutrient assimilation is therefore suggested, like the HAMGM (highly assimilable microalgae growth medium) developed by Hadj-Romdhane et al. [62] by replacing counter-ions with ammonium ion (NH_4^+). Judging from the values obtained in this study, it is very unlikely that the observed growth inhibition is solely due to salts/nutrients accumulation.

4.4 Conclusions

In an MPBR for coupled cultivation and harvesting of microalgae with permeate recycle, growth limitation was observed after 13 times of permeate recycling. It is unlikely that this was solely due to salts/nutrients accumulation. The occurrence of bioflocculation probably had an impact as well, since it lowered the number of free cells and the concentration of biomass in the bulk and the availability of nutrients. Bioflocculation could be due to medium recycle which increases the concentration of counter ions (including multi-valent cations, such as Ca^{2+} and Mg^{2+}), in combination with a high pH. As for AOM (represented by OC and TEP concentrations), influence on growth could not readily be observed, since the accumulation was rather low, which could be due to a settling of the organic compounds with the microalgae cells, as well as permeation of dissolved TEP through the membrane, or consumption by some bacteria present in the broth. However, it is clear that permeate recycle should be limited to some extent (to 13 times in our set-up and conditions). Further study on the filtration performance is necessary since membrane fouling can be the key issue that determines the feasibility of the MPBR technology. The use of a minimal medium such as HAMGM is also worth trying to further increase the amount of recycling that could be performed.

Chapter 5

Decreasing membrane fouling during *Chlorella vulgaris* broth filtration via membrane development and coagulant assisted filtration

Adapted from: Discart, V., Bilad, M.R., Moorkens, R., Boon, N., Arafat, H. and Vankelecom, I.F.J. Decreasing membrane fouling during *Chlorella vulgaris* broth filtration via membrane development and coagulant assisted filtration. Submitted.

Abstract

Membrane filtration has been reported as an interesting low-cost technique for microalgae harvesting, either in a separated process or in a coupled process such as a membrane photobioreactor. However, the filtration performance can still be improved if the membrane fouling problem can be properly managed. In this study, the improvement of the filterability of a *Chlorella vulgaris* broth both by optimizing the membrane and by dosing coagulant before filtration is investigated. For the membrane optimization, with the membranes

prepared via phase inversion, four basic membrane preparation parameters were studied, i.e., polymer concentration, time gap between casting and coagulation, addition of water as a non-solvent into the casting dope solution and the addition of polyvinylpyrrolidone as an additive to a polyvinylidene fluoride/N,N-dimethylformamide system. For coagulation, $FeCl_3$ and chitosan were tested using a polycarbonate 0.1 μm membrane. Later, some selected membranes were tested against two commercial membranes with and without coagulant dosing. The performance of the membranes was evaluated using the improved flux stepping filtration method and using a simple dead-end filtration for the coagulation/filtration study, respectively. Results show that the membrane properties could be well manipulated by the four phase inversion parameters. Also, both coagulants increased the filterability of the broth. However, when the optimized and commercial membranes were used, coagulant dosing did not significantly improve the filtration, which suggests that the coagulant type and dosing for a membrane filtration system should be optimized per membrane.

5.1 Introduction

As mentioned in the previous chapters, microalgae have long received a lot of attention because of their ability of using an inorganic carbon source and wastewater components as nutrients while producing usable biomass. The microalgal biomass can be used as a source for a variety of high-value products and – more recently – to produce biofuel [10, 97]. For the latter purpose, the required amount of biomass is huge, and production costs must be very low, much lower than the currently estimated price for a full-scale plant [1]. Therefore, a substantial reduction of production costs from different sub-processes remains a priority.

Two common types of cultivation systems to grow microalgae, i.e., open raceway pond bioreactors (up to 0.6 g/L) and closed photobioreactors (PBRs, realistically up to 2 g/L), can only achieve relatively low biomass concentrations in the bioreactor due to various limitations. The biomass requires a substantial concentration in the harvesting process before it can be processed further (drying, extraction, etc.) [31, 118]. Although the biomass can be pre-concentrated in the bioreactor to higher concentrations in a recently developed membrane photobioreactor (MPBR) system, further concentration processes are still necessary [69]. In our earlier reports [28, 27], a two stage microalgae harvesting process was proposed, primarily via low cost membrane filtration where most water is removed, followed by a high cost centrifugation. The high costs associated with centrifugation are largely reduced by decreasing the volume that has to be treated via the membrane pre-concentration.

Two membrane filtration systems, an aerated and a vibrated one, have been tested and proved to offer an interesting option to harvest *C. vulgaris* [28, 27]. However, the potential of this approach had not been optimized yet in terms of applied membranes and broth conditions. Although many studies have reported on the effect of different membrane properties on membrane filterability [138], to our best knowledge, more systematic studies focusing on membrane development aimed at microalgae harvesting are rare [40]. The opportunity to customize a membrane for harvesting a particular microalgae species is widely open due to the diversity of microalgae species with respect to cell size, cell wall chemistry and forms [76]. At the same time, a large number of membrane preparation parameters can be optimized and therefore can be customized for the desired microalgae species [70, 11, 103].

While the previous chapters monitored TEPs and their influence on several parameters in different settings (a full scale water filtration plant, lab scale microalgae filtration and MPBR cultivation system), a more practical approach of microalgae filtration system optimization was adapted in this study. In

this study, membranes were systematically developed with special emphasis on porosity while coagulants were tested to improve *C. vulgaris* broth filterability. Four basic phase inversion parameters were varied, i.e., polymer concentration, evaporation time (time gap between casting and coagulation), water (non-solvent) addition, and additive concentration, to produce 18 different membrane samples. Due to the relatively homogeneous cell size of *C. vulgaris*, membranes with a relatively large pore size (up to 1 μm) can be used. The size should be large enough to allow high permeance but small enough to retain the microalgal cell without blocking the pore. In addition, the pore density (defined as the number of pores per unit area) should be maximized to lower the local flux of the membrane [25]. The four studied parameters offer the flexibility to modulate the pore size and surface porosity. In addition to the membrane itself, the filterability of the feed can also be improved by adding a small amount of coagulant [71, 74, 134, 124]. Coagulation/flocculation in itself is a renowned way to harvest microalgae [141], but it can also be used to improve membrane filterability in both microalgae and non-microalgae applications [71, 74, 101]. For the former (harvesting by flocculation), the dosing concentration should be sufficiently high (because all the biomass has to be flocculated) which can be costly. For the latter, the dosing concentration is generally lower: there is no need to have the whole biomass coagulated. Algogenic organic matter (AOM) quenching in order to reduce its impact on fouling is enough. The flocculation experiments performed here aim to investigate the coagulation circumstances when the broth filterability could indeed be improved by dosing only a very small amount of the flocculant/coagulant – not for a complete flocculation of the biomass. Here, we assess the impact of the dosing of two coagulants ($FeCl_3$ and chitosan) on the broth filterability at different coagulant concentrations. Finally, based on the combined results of the phase inversion parameters and the coagulant optimization, three new membranes were prepared and the permeance was assessed with and without optimized coagulant dosing.

5.2 Materials and methods

5.2.1 Microalgal broth cultivation

The *C. vulgaris* (SAG, Germany, 211-11B) broth was produced from a continuous MPBR reported elsewhere [26]. *C. vulgaris* was cultured in Wright's cryptophyte (WC) medium prepared from pure chemicals dissolved in demineralized water [61]. The algae culture was grown in a plexiglas bubble column PBR, with a working volume of 25 L and diameter of 20 cm. Degassing was carried out with filtered air at a constant flow rate of 4.5 L/min. The composition of the

cultivation medium is given in Vandamme et al. [143]. The retentate was continuously collected during MPBR operation with a dilution rate of 0.3 d^{-1} and at a concentration factor of 4 to give an average retentate concentration of around 2 g/L (Figure 5.1 A). The PBR was fed using the WC medium prepared with demineralized water [61]. The concentrated broths in the retentate tank were kept aerated during the test. A small change in the broth properties is thus expected.

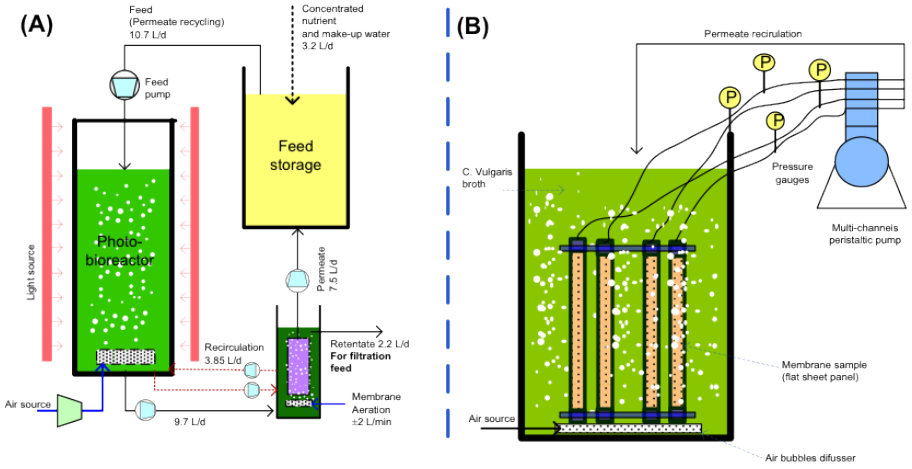

Figure 5.1: (A) Illustration of the MPBR with external filtration tank and its main operational parameters. The retentate of the MPBR was used as the filtration feed for the filterability tests. (B) Filtration set-up used for the filterability tests in which fouling control was provided via air bubble scouring.

5.2.2 Evaluation of the membrane properties

Membrane preparation

Membranes were prepared via the phase inversion method. In this method, a polymer is dissolved in a solvent (with or without additives) and cast as a thin film on a surface, after which it is submerged in a non-solvent, which causes phase inversion (i.e. the transformation of a liquid into a porous membrane) [144]. Here, the membranes were prepared from polyvinylidene fluoride as the polymer (PVDF, MW 534 kDa, Sigma-Aldrich), polyvinylpyrrolidone as additive (PVP, MW 10 kDa Sigma-Aldrich), N,N-dimethylformamide as solvent (DMF, Sigma-Aldrich) and demineralized water as non-solvent. PVDF is a

commonly used polymer for membrane preparation for several applications, and it has been shown to generate membranes quite suitable for microalgae filtration [40]. In the first stage, four series of phase-inverted membranes (each containing four to five samples to give a total of 18 membranes, see Table 5.1) were assessed. Membranes from series P, T, W and A, were prepared by varying the polymer concentration, time gap between casting and coagulation, water and additive concentration in the casting solution, respectively. The casting solution was cast with a 250 μm wet thickness and at a casting speed of 2.25 cm/s on a polypropylene non-woven support at 22-30°C (Novatexx 2471, donated by Freudenberg, Germany). After testing the impact of the four basic phase inversion parameters separately, a new set of membranes (O-series) were prepared subsequently which were expected to have a better performance based on the screening of the P, T, W and A test series (see the results in Section 5.3.1). The performance of the O-series membranes was tested against two commercial membranes: a chlorinated polyethylene membrane from Kubota (PEK) and a porous mixed matrix membrane (MMM) from Amer-Sil. These membranes were chosen because they could be used fo microalgae filtration, which is the application for which lab-made membranes were developed. The former was developed for use in membrane bioreactors for wastewater treatment, and the latter is a new membrane product aimed for common filtration. Their performance was tested with and without coagulant addition.

Membrane module assembly

Membranes were assembled into modules with an effective membrane area of 0.016 m^2 (2 sides of 8cm x 10cm). The flat-sheet membranes were fixed using a PVC frame and the inner parts of the membrane sheet edges were glued (in dry condition) together to form a small envelope using a two-component epoxy glue (UHU-Plus endfest 300, Germany). Two sheets of spacer (Intergated permeate channel, VITO, Belgium) in the interior of the module separated both active separation areas. Permeate was sucked from the module interior through the permeate line. More detailed information about the module potting is available elsewhere [24].

Filtration set-up and operation

The filtration set-up is shown in Figure 5.1 B. The filtration tests were performed in a 3 L tank. The membrane samples were submerged inside the filtration tank, where each membrane was connected through an individual line to a separate pump channel using an isoprene manifold tube in a multi-channel peristaltic pump (Watson Marlow, UK). Each line was equipped with an individual vacuum

Table 5.1: Parameters applied for membrane preparation and summary of the main corresponding membrane characteristics

Membrane series		PVDF (% w/w)	PVP (% w/w)	Evaporation time (secat)	Water (% w/w)	CWP (L/m² h bar)	Average pore diameter (µm)	Largest pore diameter (µm)	Surface porosity (%)	Contact angle (°)	TMP_F at 25 L/(m² h) (kPa)
P	P1	8	none	40	none	309	0,171	0,743	8,5	91±2	4,4
	P2	12				231	0,113	0,497	6	88±5	6,9
	P3	16				220	0,107	0,585	9,8	89±2	6,8
	P4	18				196	0,118	0,505	9,6	86±1	8,9
T	T1	14	none	15	none	143	0,134	0,775	9	85±2	16,1
	T2			30		186	0,147	0,839	9	87±2	12,8
	T3			90		143	0,113	0,483	7,5	90±3	18,3
	T4			240		190	0,138	0,815	12,8	91±1	16,2
	T5			600		887	0,16	0,839	16,1	91±2	2,2
W	W1	14	none	40	1	161	0,101	0,404	5,2	90±6	14,6
	W2				3	155	0,129	0,563	11,6	94±5	18,8
	W3				3.5	300	0,134	0,534	11,2	94±3	15,3
	W4				4	611	0,143	0,77	12,6	93±11	5,3
A	A1	14	0	40	none	208	0,101	0,505	6,3	87±3	10,7
	A2		1			163	0,08	0,311	3,6	69±3	14,4
	A3		3			268	0,113	0,573	4,7	72±2	5,2
	A4		4			1250	0,147	0,732	13,8	78±3	2,7
	A5		5			243	0,101	0,507	6,6	76±2	3,2
O	O1	8	0	40	none	447				92±4	0,6
	O2	8	4			655	n.a.	n.a.	n.a.	77±5	0,2
	O3	10	4			556				77±3	0,1
MMM	Commercial membranes					850	0,15	n.a.	n.a.	n.a.	3,0
PE_K						923	0,22				1,3

gauge. The membranes were placed above the air bubble diffuser, from which air bubbles travelled along the membrane surfaces to scour the foulants. The air was pumped continuously at a fixed flow rate of 5 L/min over all membrane samples (4-6 pieces) for each filtration batch. The flux was set by adjusting the pump rotation speed. Four to five membrane samples were tested simultaneously in one batch. They were arranged in such a way that there was 1 cm of inter-space between them. The filtrations were performed in parallel during the test to improve the comparability of the results.

Membrane characterizations

Before any filtration test started, the modules were wetted by soaking them in a 40% ethanol/water solution for 1 h, followed by compaction for a few hours at maximum pump speed, corresponding to a filtration flux of 57 L/(m^2h). The clean water permeance (CWP) was measured filtering the *C. vulgaris* broth at a fixed flux of 55 L/(m^2h) during at least 1 h. The microstructure of all membrane samples was examined using scanning electron microscopy (SEM, Philips SEM XL30 FEG with EDX dx-4i system). On the SEM images, the properties of the membranes, i.e., surface pore size, porosity and thickness, were later identified using imageJ (NIH, USA). The water contact angle of the membrane surfaces was measured using the sessile drop method (drop size of 1 μL) with a contact angle goniometer (Krüss DSA 10 Mk2). The measurement was done immediately after the water was dropped and this was performed at least 6 times for each sample.

Filterability evaluation

The basic parameters used to evaluate the filtration performance were flux (J, L/(m^2h)) and permeance (L, L/(m^2h bar)), which were calculated by using Eqs. (5.1-5.2), respectively:

$$J = \frac{\Delta V}{A \Delta t} \tag{5.1}$$

$$L = \frac{J}{TMP} \tag{5.2}$$

in which V is the permeate volume (L), t the filtration time (h), A the membrane surface area (m^2) and TMP the trans-membrane pressure (bar or kPa). The flux-stepping filtration test was used to assess the filterability of membrane samples using the improved flux-stepping filtration method (IFM) [138]. The

low flux was 5 L/(m^2h) and the flux step size was 10 L/(m^2h) (high fluxes were 15, 25, 35, 45, and 55 L/(m^2h). The step duration was 10 min. The tests were performed in six batches, one batch for each series (Table 5.1) followed by a batch, with and without the coagulant, of the optimized membranes and the two commercial membranes. The commonly applied method, the so-called critical flux measurement, compares the performance of different membranes. However, this method has rather low precision due to the significantly large step-height applied. As an alternative, the evolution of pressure drop due to the fouling resistance (TMP_F) of the membranes over the flux-stepping filtration duration was used in this study. That way, the fouling propensity of different membranes can directly be compared over all fluxes. The TMP_F is calculated using Eqs. (3-7).

$$R_T = \frac{TMP}{\eta J_v} = \frac{1}{\eta Perm} \tag{5.3}$$

$$R_T = R_M + R_F \tag{5.4}$$

$$R_F = R_{Rev} + R_{Res} + R_{Irrev} + R_{Irrec} \tag{5.5}$$

where R_F is the fouling resistance (m^{-1}), R_{Rev} the reversible fouling resistance (m^{-1}), R_{Res} the residual fouling resistance (m^{-1}), R_{Irrev} the irreversible fouling resistance (m^{-1}), R_{Irrec} the irrecoverable fouling resistance (m^{-1}) and R_M the intrinsic membrane resistance (m^{-1}). The fouling fractions in this study are defined according to Drews [47]: reversible fouling is defined as the permeance loss that can be recovered by physical measures (such as relaxation or backwash), residual fouling is fouling which remains after physical cleans and is removable by chemical maintenance cleaning (such as chemically enhanced backwashes), irreversible fouling is defined as fouling that can be recovered by the applied intensive chemical cleaning (removal by cleaning-in-place), and irrecoverable fouling is the permeance loss which remains even after cleaning-in-place procedures. Since the dynamic viscosity of the permeate (η, Pa.s) and the flow velocity (J_v, m/s) are constant over time, the filtration resistance (R_T) is proportional to TMP. Hence,

$$R_F \equiv TMP \tag{5.6}$$

$$R_M + R_F \equiv TMP_M + TMP_F \tag{5.7}$$

Where TMP_M is pressure drop due to membrane resistance. The TMP_F term is used in this study to clearly make a distinction between pressure drop due

to membrane resistance and due to the fouling layer. When TMP is used, the contribution of membrane resistance is still present and can thus lead to misinterpretation (i.e., two membranes with a large difference in L can have huge difference in TMPs at similar applied flux).

5.2.3 Coagulation filtration test

The impact of coagulant dosing on *C. vulgaris* broth filterability was investigated in a dead-end filtration cell using a 0.1 μm polycarbonate membrane (Millipore), where all the fluid passes through the membrane and all particles larger than the pore sizes of the membrane are retained at its surface by pressurizing the liquid feed perpendicular to the membrane surface under stirring. Two common coagulants were applied: $FeCl_3$ and chitosan (both from Sigma-Aldrich). After being dosed with variable concentrations of coagulant (0, 2, 5, 10, 20 and 50 ppm for $FeCl_3$ and 0, 2, 5, 10, and 20 ppm for chitosan), the broth samples (3 per dosing) were first stirred strongly during 2 min, after which it was stirred gently at 50 rpm for 15 min, before being placed in the filtration cell (3 filtrations simultaneously). The broth samples were filtered through the polycarbonate membranes at a fixed pressure of 1 bar, for variable times until 60% of feed had permeated. The filtration performance was evaluated as (1) the normalized average flux after 15 min until 60 min of filtration and (2) the normalized flux decline after 15 min until 60 min of filtration. Membranes from the O-series and the two commercial membranes were similarly tested with 10 ppm of chitosan.

5.3 Results and discussion

5.3.1 Membrane screening

Membrane characteristics

The four basic parameters that were varied during the synthesis of the membrane samples, were found to have a significant impact on the resulting membrane properties (see Table 5.1). All membranes were within the ultra- and microfiltration pore size range, with pore sizes ranging from 0.08 to 0.17 μm. Therefore, most of the biomass was retained (*C. vulgaris* cells are 1-2 μm in size), as also visually observed during the filtration test. No quantitative analysis was performed to confirm this. The microstructures of the surface of the membranes from P-series, recorded using SEM, are shown in Figure 5.2. On the images (also refer to Table 5.1), the trend of decreasing pore sizes

with increasing polymer concentrations is clear, as could be expected [103, 160], except for P4. It could be that the high concentration in the P4 polymer solution gives a lower stability of the solution, which in combination with the H_2O vapor in the lab causes a faster demixing and thus a more porous sample.

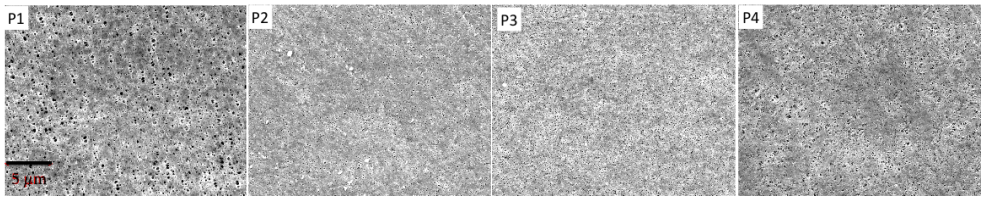

Figure 5.2: SEM images (all share one scale) of membrane samples from group P, showing a decreasing pore size with the increase of polymer concentration (except for sample P4).

The behavior of the casting film during a prolonged exposure to air before coagulation is interesting. Usually, when a volatile solvent is used, it evaporates while water from the humid laboratory air condenses onto the top of polymer film which is cooling down at that moment as the heat of evaporation is dissipating [47]. Since DMF is not considered volatile (boiling point of 152-154 °C), the condensation of water vapor on top of the polymer film might become pre-dominant. Therefore, it brings the phase inversion process more toward more instantaneous demixing [87]. Under a humid atmosphere, extending the exposure time between casting and coagulation allows more adsorption of water vapor from the air on the top of the cast film [87, 56]. Therefore, it changes the composition of the casting solution, reduces the polymer concentration locally (only on the top) and at the same time induces the phase inversion process toward more instantaneous demixing [87]. This renders the membranes more porous, as shown for the T-series (T1-T5) in Table 5.1. The permeance tends to increase as the time gaps between casting and coagulation increase, as also observed from the increase in average pore diameter from 0.13 to 0.16 μm at increasing time gaps from 15 to 600s. Addition of water as non-solvent to the casting solution also increases the average pore size from 0.087 to 0.143 μm (Table 5.1), which is in line with the corresponding increase in the CWP. Addition of water basically shifts the casting solution composition in a three phase polymer/solvent/non-solvent diagram closer to the binodal boundary. Therefore, it is expected that with higher water content the phase inversion starts earlier, unless the composition does not reach the meta-stable region [160]. The effect of PVP addition on the pore size and CWP in this study is somewhat unclear (Table 5.1). No significant change in the pore size was found, nor any trend in the CWP. However, the addition of PVP significantly lowered

the contact angle, compared to membranes without PVP, irrespective of the PVP percentages. This indicates that PVP addition rendered the surface more hydrophilic and the PVP thus partly remained entangled in the polymer matrix, despite its hydrophilic nature and solubility in water.

Flux stepping filtration

The TMP profiles of the four membrane series during the IFM filtrations is shown in Figure 5.3 (for the full TMP vs. time graph, see Figure C.1 in the appendix). Only TMP_F data below 20-30 kPa (depending on the pump channel) are presented since the data are not accurate beyond that value due to pump limitations. The filtration test was performed once for each membrane serie. As shown in Figure 5.3, generally the TMP_F was almost zero at very low fluxes, indicating an almost complete absence of membrane fouling. The TMP_F then increased almost linearly with the increase in flux, but as the fluxes increased further, the TMP_F rise became exponential, indicating an accelerated membrane fouling rate. The applied flux beyond which the linear relationship between applied flux and TMP disappears is commonly called the critical or (sometimes) threshold flux, describing the boundary between low and high fouling rate regimes [8, 52]. In many cases, the threshold value is used to compare the filterability of different membrane/feed systems, but here we directly used the TMP_F as evaluation parameter because it provides a higher accuracy. It is worth noting that the precision of the threshold flux is strongly influenced by the applied step height. When the step height is too high, the precision is low, and vice versa. When applying small step heights, a substantially longer filtration is required since more steps are necessary before reaching the maximum applied flux in our measuring system. It would also prolong the testing duration, risking changes in the broth properties. For some membranes, the TMP_F lowered at high fluxes, after having reached a maximum at 25 (for the T1, T2, T3 and A2 membrane) or 35 L/(m^2h) (for the W1 and W2 membranes) (Figure 5.3 and C.1). This trend exists due to pump limitations. As soon as the TMP reached maximum allowable values (20-30 kPa), the pump could not maintain a constant flux operation anymore and compensated the fouling at high fluxes by reducing the flow rate instead of increasing TMP. This problem could not be avoided.

The effect of each of the four basic membrane preparation parameters on the performance of the membrane was evaluated separately (Figure 5.3 and Figure C.1 in the appendix). To illustrate these effects, an additional plot comparing all TMP_F-values at 25 L/(m^2h) flux is given in Figure 5.4. The trend for the P-series in all these figures was clear: the lower the polymer concentration, the lower the pressure over the membrane at an equal flux. This is also in

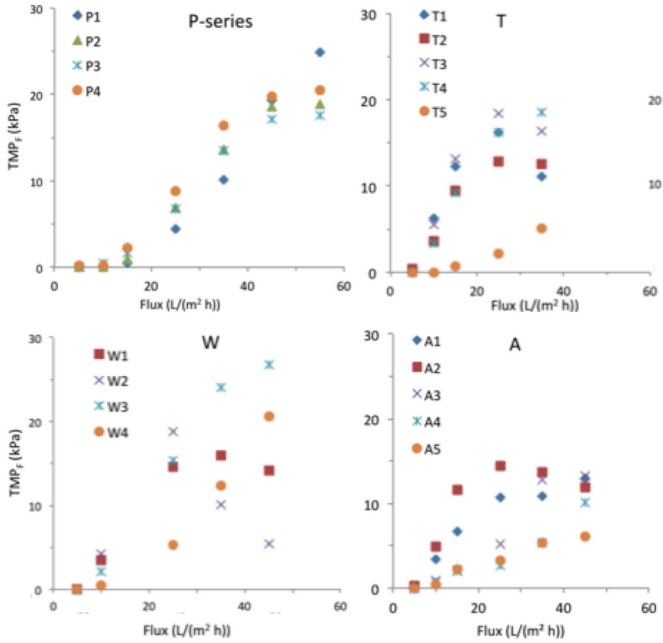

Figure 5.3: Flux stepping profile of membranes from left to right: P-, T-, W- and A-series (P: polymer concentration; T: air contact time; W: water addition; A: PVP addition). Microalgae broth was used as feed. The filtration test was performed once for each membrane serie. The data points represent the TMP_F at the end of each flux step. All figures share the same y-axis title.

agreement with the trend in pore size, shown in Table 5.1. This means that the concentration of polymer should be kept as low as possible.

For the T-series, the trend was not as clear as for the P-series. For the air contact times of 15 to 240 s (T1-T4), there was no relationship between the TMP_F-values and the contact times (illustrated in Figure 5.4) and the relationships between the different TMP_F-values change over the course of the experiment (after a longer filtration time and at a higher flux). Only the longest exposure time of 600 s (T5) gave at the same time a higher average diameter, a higher surface porosity (Table 5.1), as well as a lower TMP_F (Figure 5.4). An explanation for this could be that there is a tipping point somewhere between 240-600 s where the structure of the membrane changes. A leak in the membrane could also have explained the result, but there were no microalgae found in the permeate. The concentration of water in de polymer solution did not give a clear effect either,

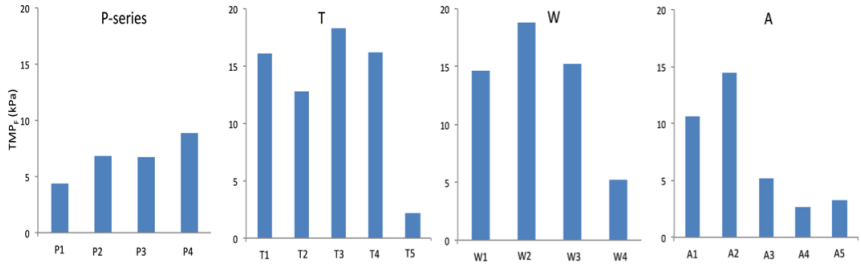

Figure 5.4: Summary of the final TMP values at the 25 L/(m^2h) flux. All figures share the same y-axis title. P: polymer concentration; T: air contact time; W: water addition; A: PVP addition.

except, again, for the last sample (with the highest water concentration). For water concentrations from 1 to 3.5% (W1-W3), no trend could be found. For the 4% concentration (W4), the membrane initially gave a low TMP_F-value, but at higher fluxes the TMP_F-rise was much higher than for the other membranes (Figure C.1). This might be explained by the larger pores of the membranes (both the mean pore size and the largest pore size), which would give lower pressures at the start of the measurement and a faster rise of the pressure at higher fluxes because of pore blocking. However, this was not observed for the T5 (600 s) membrane, which also has very large pores (Table 5.1). Perhaps these were large enough to let the pore blocking substances pass. The addition of PVP seemed to have a beneficial effect on the performance of the membranes, although there was no straightforward link between the amount of PVP added and the improvement of performance. It is possible that there is an optimal concentration of PVP (in this cas 4%, A4). This could explain why the 4% performs better than the membrane with 5 and 3% addition (A3 and A5). For the 4% PVP membrane (A4), despite a similar pore size and porosity as the W4 membrane, the faster rise of pressure at higher fluxes was not observed (Figure C.1), so there was no indication of pore blocking in this case. The reason for this discrepancy is unknown. In any case, membranes with sufficient amount of additive in the casting solution (3-5%, A3-A5) performed better than the ones with little or no additive (0-1%, A1-A2) (Figure 5.4, Figure C.1). One reason for this could the higher hydrophilicity, although this did not seem to change the differential performance of the O1 and O2 membranes (Section 5.3.3).

In all, the parameters of the PVDF membranes that gave the clearest link with an improved filtration performance were the polymer concentration (as low as possible) and the additive concentration (around 3-5%). Therefore for the following tests, somewhat “optimum” membranes were made (membranes

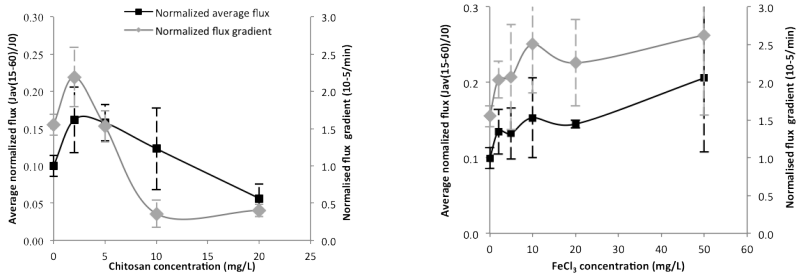

Figure 5.5: Impact of coagulant dosing on the filterability of a 0.1 μm polycarbonate membrane.

O1-O3, see Section 5.2.2) which were expected to perform better than any of the previous lab-made membranes.

5.3.2 Coagulation optimization of the microalgae broth

Since AOM, which is mostly negatively charged, is involved in membrane fouling during microalgal broth filtration [28, 27, 142], the elimination of that AOM by neutralizing the negative charges with coagulants to form larger agglomerates seems to be an effective way to curb its impact on membrane fouling. Figure 5.5 (right panel) shows that increasing the $FeCl_3$ dose increases the flux and the flux gradient. Both parameters give an idea of the filterability of the broth: while the rise in flux is wanted, the rise in flux gradient is not wanted, because this indicates a faster clogging of the membrane. In this case, the flux gradient generally did not rise more than the average flux (except at the 10 mg/L dosing). Dosing 10 mg/L of $FeCl_3$ improved the flux by about 50%, while using 50 mg/L $FeCl_3$ yielded almost 100% improvement. At the applied dosing, no flocculation of the biomass was observed. In fact, since flocculant/coagulant can also be a major cost contributor, the dosing concentrations should be minimized, while still offering a proper filterability improvement.

Chitosan showed an interesting behavior as a filterability enhancer (Figure 5.5). The filterability increased when dosing a concentration of 2-5 mg/L, but at higher concentrations it dropped, even below the starting chitosan-free case. The flux gradient, on the other hand, reached a minimum at 10 mg/L. Because there is a dosing for maximum flux and a dosing for minimum flux decline, there is probably a optimum dosing of chitosan between 5 and 10 mg/L. These low concentrations could indeed significantly reduce the coagulant costs and make it very interesting as a filterability enhancer of *C. vulgaris* broths,

when aimed for bulk final production (i.e. biodiesel). The mechanism behind this decreasing filterability trend with chitosan dose is still unclear, possibly because of the formation of a very thin additional filter cake on the membrane surface from the settled AOMs. The chitosan dose at peak filterability values was obtained at a pH of 7 ± 0,2. It should be noted that the optimal dosing would be dependent on the pH, because of the pK_a value of chitosan of 6.5. This means that at pH 6.5 half of the amino groups will be protonated and the other half deprotonated. Because chitosan is used here to bind negatively charged particles, like microalgae, TEPs and EPS, it will be most effective in its fully protonated form, hence a low pH, with the limitation that the negatively charged particles should not become protonated themselves.

5.3.3 Optimized system: membranes and coagulant assisted filtration

The two membranes (O2 and O3) were prepared by selecting the optimum basic parameters (polymer concentration and additive concentration) from Section 5.3.1. Just like for some of the initial membranes (see higher), the TMP_F lowers at high fluxes for some of the membranes, after having reached a maximum at 45 L/(m^2h) (for the MMM membrane with and without coagulant and the O1 membrane without coagulant) (Figure 5.6 and C.2). The reason is the same as to the ones explained earlier (pump limitation). The data for the low flux (5 L/(m^2h)) showed a very low irreversible fouling rate (Figure C.2) over the course of the experiment, except for the MMM membrane, especially after coagulant addition. In any case, all membranes showed a good biomass rejection, as observed visually. Their performance was even better than that of the two tested commercial membranes (MMM and PEK). This finding suggests that one can customize a membrane for microalgae harvesting, instead of using existing membranes that were mostly developed for other purposes. Furthermore, considering the diversity of microalgal species, the membrane customization should probably be done per species. It is worth mentioning that despite the clear advantage with respect to the CWP and the lower contact angle, the performance of membrane O2 is almost similar to O1. It seems that the wetting step prior to filtration was sufficient to wet most of the pores. It also indicates that the hydrophilic nature of the membrane is less important in membrane fouling for reported systems.

In terms of impact of coagulant dosing, 10 ppm chitosan was selected because of an initial flocculation test. The flocculation test was performed with 5 and 10 mg/L chitosan on the algae suspension, and 10 mg/L gave a better floc formation. The results in Figure 5.6 are somewhat contradictory to those obtained earlier. Intuitively, one would expect that addition of coagulant would improve the

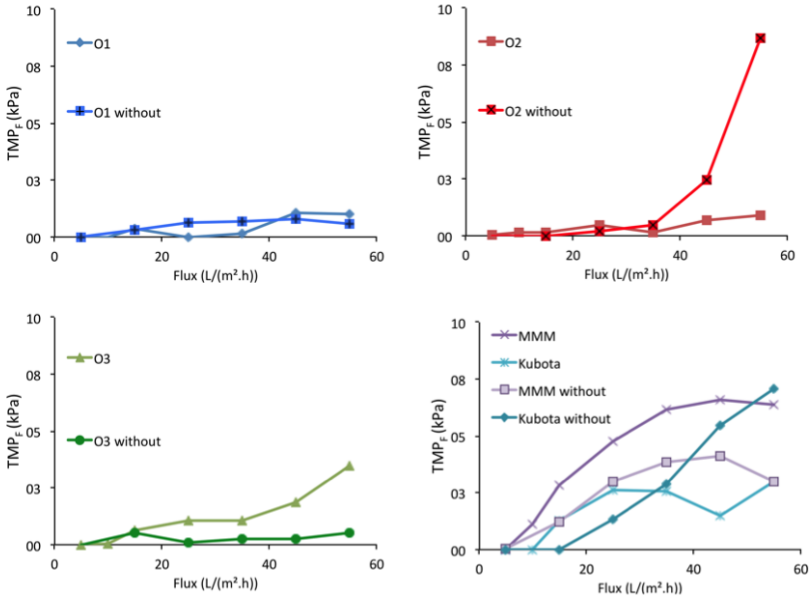

Figure 5.6: Flux stepping profiles of the selected membranes (O1-O3) and two commercial membranes (MMM from Amer-Sil and PE from Kubota) without and with chitosan (10 ppm) as coagulant.

performance of any membrane. However, no clear trend was observed in this study. The coagulant dosing had little effect on membrane O1, it improved filterability of O2 at higher fluxes, it lowered the filterability of membrane O3 and the MMM, and, depending on the flux, for the Kubota membrane (only beneficial at higher flux). Therefore, no straightforward conclusion can be withdrawn from the results. Most probably, the optimum coagulant dosing is also a function of the membrane, which suggests that the coagulant type and dosing optimization should be customized for each membrane. A more detailed study is required to unravel this finding, which possibly is related to membrane charges or polarity.

5.4 Conclusions

The significant improvement of *C. vulgaris* broth filterability via both optimizing the membrane and dosing coagulant has been demonstrated in this study. The filterability could be improved to some extent by applying highly porous

membranes, which were obtained by casting the membrane from solution with a lower polymer concentration, by implementing longer time gaps between casting and coagulation or by adding water or PVP to the casting solution. By combining these parameters, a membrane with a much-improved filterability could be obtained for the *C. vulgaris* system. During the screening and optimization test using 0.1 μm polycarbonate membranes, dosing coagulants ($FeCl_3$ or chitosan) improved the filterability of the broth. However, no clear advantage of coagulant dosing was observed for the optimized PVDF-based membranes (O-series), which suggests that coagulant type and concentration are probably a function of membrane properties, thus, leaving room for case-by-case optimization.

Chapter 6

Conclusions and future perspectives

6.1 Conclusion

6.1.1 Defining TEPs

TEPs have only quite recently gained attention in biofilm and membrane research. However, the particles are still rather vaguely defined. The original definition is "particles retained by PC filters with a pore size of 0.4 μm and stainable by Alcian Blue (AB)", which implies that they consist of (or contain) acid polysaccharides and are rather large (although the dynamic nature of the particles implies that they can vary in size according to the circumstances). More specifically, TEPs are sticky, transparent, gel-like, sugary particles. The main difference with EPS/SMP (extracellular polymeric substances/soluble microbial products) is that they are not necessarily formed within a biofilm context, but are rather free floating particles that can be formed by the coagulation of soluble polysaccharides. Although the precursors are derived from organic systems, these precursors can form the larger TEPs through abiotic processes. Shear forces working on a sample can thus cause new TEPs to be formed from dissolved precursors or they can break down existing TEPs.

The emergence of alternative measurement methods only added to the vagueness surrounding the particles. While trying to simplify the original method, which is highly sensitive, but also labor-intensive and complex, they accentuated the

constrictions of using the term "TEPs". When comparing different studies, it should thus always be kept in mind that there might be different methods involved leading to different results. A classification as proposed in Chapter 1 is useful to keep a clear view on what is exactly measured in each situation. Also in this PhD, several methods were used in the different chapters (still all using AB), since one method is better adapted for the low concentrations that were typically found in the ultrafiltration (UF) purification plant, while another is more suited for the long-term screening tests of microalgae broth where much higher concentrations were present.

6.1.2 Role of TEPs in surface water purification and microalgae cultivation

Before the start of this PhD, several studies had shown a possibly large impact of TEPs (determined by different methods in different studies) on membrane fouling. These studies generally focused on water with a relatively high fouling load, such as seawater and activated sludge. The present study aimed at expanding the knowledge about the possible influences of TEPs to other systems. In general (over the broad areas going from water purification to microalgae cultivation and UF), the impact of TEPs as a separate factor seemed limited, although some interesting trends were found in this PhD.

In an industrial UF surface water purification plant (Chapter 2), the feed water was extensively pretreated before being sent over the UF membrane. Despite this pretreatment, irreversible fouling caused the transmembrane pressure (TMP) to rise significantly in a few months. Since there was a low fouling load, microalgae and TEPs seemed like the most probable causes for this TMP rise. The bad correlation between TEP-concentrations and the TMP, and a membrane autopsy, led to the conclusion that TEP concentrations were found to be less important for membrane fouling in comparison to the presence of the microalgae themselves. The TEP method used here detected the acidic polysaccharides (stainable by AB) retained by PC filters with a pore size of 0.1 μm. It could thus be concluded that other microalgae-derived substances, stainable by other methods (the Dubois assay for example), or smaller than 0.1 μm, are important here. Indeed, during membrane autopsy, no microalgae (or other microbial) cells were detected on the membrane, nor were TEPs. These results are not consistent with literature, where TEPs were suggested to cause a significant increase in membrane resistance due to several kinds of fouling. For example, Kennedy et al. [79] indirectly suggested that TEPs, in their study present at concentrations up to 10 times as high as the concentrations at the Gavers plant, are related to an increase in membrane resistance due to reversible and irreversible fouling. Bar-Zeev et al. [14] found a critical role of microgel particles,

such as TEPs and protobiofilms, in facilitating biofilm formation. However, they did not provide the concentration at which the contribution became significant. In another study, Bar-Zeev et al. [15] showed that at concentrations of around 250 μg/L (twice the maximum concentration found in the Gavers feed water), TEPs could significantly promote biofilm formation. Interestingly, in a followup study, they showed that this biofilm formation was irrespective of the present microbia being 98% inactivated or fully active [20]. In all, most studies of water purification where a high correlation was found or suggested between TEPs and membrane fouling, much higher TEP concentrations were in play.

The next studies of this PhD focused on the role of TEPs in the cultivation of microalgae. No reports existed yet on the influence of TEPs on the filtration of microalgae cultivated for commercial reasons, while in natural environments, the majority of the TEP precursors is produced by microalgae. In the first of these microalgae-related studies (Chapter 3) the contribution of the particles to membrane fouling during dead-end filtration of distinct *C. vulgaris* broth solutions was evaluated. The TEP method used here was roughly the same as in the first study: it determined components stainable by AB and retained by PC filters with a pore size of 0.1 μm. When using UF membranes for the microalgae filtration, the higher pressure pushed all cells in a dense cake layer which determined the permeance, and where no distinction could be made between microalgae cells and their products. However when using low-pressure microfiltration membranes, soluble compounds, TEPs and carbohydrates, had a differential influence on the flux decline. However, it was not clear for TEPs alone. There was thus no proof that TEPs as a subgroup of carbohydrates were more important than other soluble compounds in creating the membrane fouling, although they are regarded as orders of magnitude stickier than other particulates [19, 41] . This is different from the study of De la Torre et al. [41], where TEPs fitted better than carbohydrates in all cases when plotted against the critical flux. In their study, although no single universal fouling indicator could be found, they found using multivariable analyses that the critical flux values for 95% of the data could be explained using four parameters measured in the activated sludge. Two of those four parameters were bound and soluble TEPs. A reason for this difference could again be a difference in concentration of TEPs or carbohydrates. Although the concentrations were not mentioned in that study, in another study [42], TEP concentrations were mentioned to be over 10 mg/L (comparable to the concentrations in Chapter 3) and carbohydrate concentrations around 10 mg/L (much lower than the concentrations in Chapter 3).

In a second microalgae-related study (Chapter 4), TEPs were studied from another perspective together with some other microalgae broth components: not as possible membrane foulants, but as components of an integrated cultivation

system (MPBR with permeate recycle) where it could have impact on microalgae growth and productivity itself. In the end, the accumulation of TEPs (here measured as AB-stainable products in the solution) in this system was found to be limited at the end of the observation period. However, together with the accumulation of non-limiting nutrients, the occurrence of bioflocculation (due to high pH), limited microalgae growth. Permeate recycle should thus be limited to some extent (e.g., to 13 times for the presented set-up and conditions).

While in chapters 1-4, TEPs and their influence on several parameters in different settings (a full scale water filtration plant, a lab scale microalgae filtration and MPBR cultivation system) were studied, a more practical approach of microalgae filtration system optimization was adapted in Chapter 5). In this study, membrane development specifically aimed for *C. vulgaris* harvesting was attempted, to prepare a membrane that functions optimally with the specific cell size and cell wall chemistry of this microalgae. Only 4 membrane preparation parameters were varied and this already gave significant improvement of *C. vulgaris* broth filterability as compared to standard and commercial membranes. The addition of some coagulants improved the filterability further, although this was not clear for all membranes.

In general, the impact of TEPs thus seemed quite limited, whether in relation to membrane fouling during water or microalgae filtration or in relation to impact on growth of microalgae in an MPBR reactor. Of course, these are only case studies: it could be that in a UF membrane purification plant with slightly different conditions, for example with different temperatures, different (microalgae) species, TEPs could emerge as a determining factor. Likewise, for microalgae cultivation, different cultivation parameters (e.g. a different composition of the growth medium) could generate TEPs with different characteristics or other relative amounts (relative to other algogenic organic material (AOM)). To be more conclusive about the role of TEPs in several systems and under wider conditions, further research is still needed.

In all, TEPs are a group of ubiquitous aequous particles that are not easy to define or quantify. Their role in the studied systems does not seem as large as was expected initially, based on earlier reports.

6.2 Future perspectives

6.2.1 TEP detection method

The differences between the various TEP detection methods have been thoroughly discussed in the first Chapter, and a possible classification of TEPs

was presented. There are still several possibilities left regarding the improvement of these methods. For example, the calibration of the Passow & Alldredge and Villacorte methods could be further explored. As mentioned before, the calibration is very difficult because of the very low concentration levels on one hand, and of the dynamic properties of the standard xanthan gum solution on the other hand. There have been several attempts to try to simplify the calibration method (e.g. by using total organic carbon analysis instead of weighing, or by abandoning the traditional calibrations and focusing on standardization of the dye solutions). Still, more research should be performed to assess the repeatability of the measurements when using a certain calibration method. A possible strategy could be to compare the obtained TEP concentrations from measurements using different calibration methods on the same sample. Some other, small changes to the method could also be tried, such as the use of lower AB concentrations, while adding a larger volume of the dye solution. Improved standardized washing of the sample before staining is also something to be considered, since soluble compounds, such as salts, could interfere with the staining. As mentioned in the original Passow & Alldredge work, AB also stains the PC filter itself, which necessitates the use of filter blanks. Thornton already experimented with different filter materials for their acid polysaccharide measurements, but it could be useful to try these also as variations on the original method, which continues to be the most sensitive method until now.

Also the Arruda Fatibello method, despite being less sensitive, and being the easiest method in practice, deserves some further investigation. One aspect is the effect of pH on protein/nucleic acid staining, since the method uses a higher pH than the original method. For this, BSA and DNA calibration curves could be constructed.

Furthermore, some more comparison between the main methods is necessary, for example by measuring TEPs with the Arruda Fatibello method before and after filtration with a polycarbonate (0.4 μm) membrane.

Alternatively, an attempt could be made to develop a whole new method, where sensitivity and ease of use are combined. An example of such a method could consist of the following steps: the sample with TEPs is poured in a filtration cell with a 0.05 μ membrane. Dissolved components are removed by upconcentration of 100 mL to 10 mL. This retentate is then resuspended to 100 mL and again upconcentrated to 10 mL. After this, AB is added to the 10 mL of solution, filtration is resumed and AB in the filtrate is measured. At the same time, the filtration time can be monitored as a measure of the pollution rate. This way, contaminants will be less of a problem for the method and the sensitivity can be improved by the upconcentration step.

Very recently, Villacorte et al. [149] developed a new method which allows

measurement of both TEP and their colloidal precursors without the interference of salinity: TEPs and their precursors are first retained on a 10 kDa membrane, rinsed with ultra-pure water, and re-suspended in ultra-pure water by sonication, then stained with AB, followed by exclusion of TEP-AB complexes by filtration and absorbance measurement of residual AB. The concentration is then determined based on the reduction of AB absorbance due to reaction with acidic polysaccharides, blank correction and calibration with Xanthan gum standard. The extraction procedure allows concentration of TEPs and their precursors which makes it possible to analyse samples with a wide range of concentrations (down to <0.1 mg Xeq/L).

6.2.2 TEPs and membrane based systems

TEPs and filtration

To determine whether TEPs with different characteristics or other relative amounts are indeed present in different conditions, TEP concentrations could be determined in other cultures of microalgae (e.g. pure cultures of *Scenedesmum obliquus*, *Phaeodactylum tricornutum* and *Nannochloropsis salina*; mixed cultures of natural algae from seawater and from fresh water). TEPs could be separated from the microalgae by centrifugation, after which the differential influence of the TEPs coming from different sources on filtration and fouling could be assessed for the filtration of microalgae (high concentrations) and for water purification (lower concentrations). This way, it could become clear where conditions indeed exist where TEP monitoring and control would be important for preventing membrane fouling.

Also the influence of membrane characteristics on filtration could be further tested by further varying the pore size, membrane charge and hydrophilicity of the membranes. The deposition itself of TEPs on different membranes could be examined, as well as the influence of TEPs on biofilm formation by using for example *Pseudomonas aeruginosa* and *Escherichia coli* as model organisms. *Pseudomonas aeruginosa* is one of the renowned biofilm forming pathogens, that are also present in a lot of different environments, including membrane bioreactors [161]. After the filtration experiments, the TEP-containing solutions showing the largest and lowest impact on membrane fouling could be further characterized. The characterization could consist of the determination of the presence of bacteria, the amount of protein and carbohydrates and the charge density of the solution. By gaining insight in the interactions between TEP, algae, bacteria and the membrane surface, specific solutions could be searched to minimize the influence of TEP on membrane filtration.

TEPs and microalgae

In Chapter 4, the influence of TEPs on microalgae growth was studied for the MPBR system, but since membrane fouling can be the key issue to determine the feasibility of the MPBR technology, the influence of the particles on fouling in this system should also be addressed. The use of a minimal medium, such as HAMGM (Highly assimilable microalgae growth medium), in the MPBR system is an interesting option that deserves further exploration, specifically with regard to its effect on biomass productivity, TEP production and the filtration performance itself. Furthermore, the effect of growth conditions and TEP production on specific aspects of the biomass production, such as the lipid and protein content of the microalgae cells, is also of importance for future applications.

Anti-TEPs

In cases where TEPs do have a severe impact, anti-TEP measures should be investigated. The type of anti-TEP measures, preventive or curative, will probably depend on the system considered. For example, for microalgae where low stress causes them to secrete less AOM (such as found by Surosz et al. [132]), the straightforward way to prevent TEP formation would be to lower the stress level of the microalgae. However, there could be a tradeoff, since it has been shown that microalgae increase the proportion of triglycerides produced upon nutrient starvation and other environmental stresses [58]. When prevention is not feasible, curative measures could be the development of TEP caption techniques (such as the "TEP Trap" [131]). The use of adapted coagulants is another curative option: in the study of Kennedy et al. [79], fouling as well as TEP concentrations could be lowered in a UF filtration system by in-line coagulation pre-treatment with 1.5 mg Al^{3+}/L. Of course, other coagulants could be considered: the ideal flocculant could depend on the system (dose required, toxicity level allowed, versatility of the system, ...), as well as on cost of the flocculant itself.

6.2.3 TEPs and biofilm formation

TEPs don't seem to be very important in fouling in the investigated systems, but they could be of high importance as an invisible food source in water systems. Van Nevel et al. [139] investigated whether these particles could be important in drinking water systems: since earlier studies had shown that sand filtration and even UF filtration systems cannot always remove TEPs from the

feed water, there seems to be a possibility of TEPs reaching the drinking water in systems where no reverse osmosis is present in the treatment line. Since TEPs promote biofilm formation, this could have safety implications for the drinking water distribution network. In that study, in none of the three investigated installations, TEP reached the final drinking water distribution system at significant concentrations, despite the absence of reverse osmosis in two of the installations. There are many other situations where biofilms, and thus their promotion by TEPs, are important. Especially situations are to be considered where microorganisms are supposed to be present in relatively low concentrations (unlike an membrane bioreactors or microalgae broth), while their products (EPS, SMP, TEP, AOM) could still float around in a large amount. Thus TEPs could promote biofilms in other water based systems, such as cooling towers, swimming pools, domestic water systems (dishwasher and washing machines) and showers, ice making machines, refrigerated cabinets, whirlpools, hot springs, fountains, dental equipment, automobile windshield washer fluid and industrial coolant. In each of these systems, it could be an interesting option to look for the possible influence of TEPs (which would probably be higher for outdoor fountains than for well-maintained showers). If some coagulant adapted to the system (for example for water cooling systems, the toxicity of aluminum chloride would be less important than for outdoor fountains where animals and children could drink from them) could neutralize TEPs, it might prevent biofilm formation to some extent and diminish waterborne infections. Such waterborne infections do not always imply ingestion of the water. Legionella species can form biofilms in water systems and spread subsequently through the air for several kilometers.

Also, livestock breeding often involves use of antibiotics to prevent infections, but the monitoring of water purity and prevention of biofilm formation on water containers could be a more sustainable approach. Alternatively, it could be an option to promote biofilm formation by the "beneficial" bacteria, such as *Selenomonas ruminantium*. Since one of the reasons for high antibiotics use in animal agriculture is to lower the amount of energy taken up by the bacteria instead of the animal itself, there are some other benefits to be found in this approach: some bacteria promote the total tract digestibility of feed components, and have been shown to increase weight gains and milk production [53]. At the same time, specific microalgae which produce TEPs and have a nutritional value, could be added.

Appendix A

Appendix of Chapter 2: TEPs and membrane fouling in a full-scale UF plant: feed parameters analysis and membrane autopsy

Table A.1: Correlations between feed water parameters (p-values).

	cTEP	TEPtot	NH4	Temp	Fe	Chla pond	Chlb pond	Chlc pond	Chltot pond	Pheo pond	Chla canal	Chlb canal	Chlc canal	Chltot canal	Pheo canal	NO3	SS	Ni	Mn	Cu	SiO2	Zn	Total N
pTEP	***	***				*		***	**	*	***		***	***	*	***	***						
cTEP	1	***				***		***	***	**			***				***						
TEPtot		1				***		***	***	**			***				***						
NH4			1																***				
Temp				1							***		*	***	***						(***)		
Fe					1														***				
Chla pond						1			***	***							***						
Chlb pond							1																
Chlc pond								1	***	***			***	***			***						
Chltot pond									1	***							***						
Pheo pond										1					*		***						
Chla canal											1		***	***	***		*						
Chlb canal												1											
Chlc canal													1	***	***								
Chltot canal														1	***	*	*						
Pheo canal															1								
NO3																1							***
SS																	1						
Ni																		1					
Mn																			1				
Cu																				1			
SiO2																					1		
Zn																						1	

*** : p<0.01; ** : p<0.02; * : p<0.05; TEPtot = total TEP concentration

Table A.2: Correlations between feed water parameters (R^2-values).

	cTEP	TEPtot	NH4	Temp	Fe	Chla pond	Chlb pond	Chlc pond	Chltot pond	Pheo pond	Chla canal	Chlb canal	Chlc canal	Chltot canal	Pheo canal	NO3	SS	Ni	Mn	Cu	SiO2	Total N
pTEP	0.54	0.77				0.42		0.57	0.46	0.48	0.67		0.59	0.67	0.43	0.46	0.56					
cTEP	1	0.95				0.49		0.65	0.63	0.59			0.53				0.76					
TEPtot		1				0.64		0.77	0.69	0.60			0.61				0.79					
NH4			1																0.52			
Temp				1							0.544		0.39	0.54	0.55						-0.88	
Fe					1														0.47			
Chla pond						1			0.73	0.95							0.69					
Chlb pond							1															
Chlc pond								1	0.51	0.60			0.52	0.40			0.55					
Chltot pond									1	0.94							0.50					
Pheo pond										1					0.44		0.69					
Chla canal											1		0.83	0.98	0.87		0.374					
Chlb canal												1										
Chlc canal													1	0.87	0.63							
Chltot canal														1	0.85	0.36	0.37					
Pheo canal															1							
NO3																1						0.96
SS																	1					
Ni																		1				
Mn																			1			
Cu																				1		
SiO2																					1	

Table A.3: Correlations between filtration performance and feed water parameters (p-values).

		IFR_L				IFR_R			
		Rack 1	Rack 2	Rack 3	Rack 4	Rack 1	Rack 2	Rack 3	Rack 4
IFR_L	Rack 1	1							
	Rack 2		1						
	Rack 3		***	1					
	Rack 4				1				
IFR_R	Rack 1	***			**	1			
	Rack 2		***	***		**	1		
	Rack 3		***	***			***	1	
	Rack 4								1
	pTEP		*	*					
	cTEP						*		
	TEPtot		*				*		
	NH_4								
	Temp								***
	Fe			*			*		
	$chla^{POND}$	**				***	*	*	
	$chlb^{POND}$								
	$chlc^{POND}$								
	$chltot^{POND}$	**				***	*	*	
	$Pheo^{POND}$					**			
	$chla^{CANAL}$								
	$chlb^{CANAL}$								
	$chlc^{CANAL}$								
	$chltot^{CANAL}$								
	$Pheo^{CANAL}$								
	NO_3								
	SS			**				*	
	Ni								
	Mn								
	Cu								
	SiO_2								***
	Zn								
	Total N			*					

*** : p<0.01; ** : p<0.02; * : p<0.05; TEPtot = total TEP concentration

Table A.4: Correlations between filtration performance and feed water parameters (R^2-values).

		IFR_L				IFR_R			
		Rack 1	Rack 2	Rack 3	Rack 4	Rack 1	Rack 2	Rack 3	Rack 4
IFR_L	Rack 1	1							
	Rack 2		1						
	Rack 3		0.79	1					
	Rack 4				1				
IFR_R	Rack 1	0.90			-0.57	1			
	Rack 2		0.88	0.81		0.489	1		
	Rack 3		0.86	0.86			0.87	1	
	Rack 4								1
	pTEP		0.52	0.52					
	cTEP						0.50		
	TEPtot		0.53				0.52		
	NH_4								
	Temp								-0.81
	Fe			-0.46			-0.48		
	$chla^{POND}$	0.57				0.62	0.47	0.51	
	$chlb^{POND}$								
	$chlc^{POND}$								
	$chltot^{POND}$	0.56				0.63	0.52	0.53	
	$Pheo^{POND}$					0.56			
	$chla^{CANAL}$								
	$chlb^{CANAL}$								
	$chlc^{CANAL}$								
	$chltot^{CANAL}$								
	$Pheo^{CANAL}$								
	NO_3								
	SS			0.61				0.52	
	Ni								
	Mn								
	Cu								
	SiO_2								0.82
	Zn								
	Total N			0.48					

Appendix B

Appendix of Chapter 3: Role of TEPs in membrane fouling: *Chlorella vulgaris* broth filtration

Table B.1: Coefficient correlation between sample variables and filterability parameters.

		Filtration parameters								
	Sample variables	$L_{-Lost>t15}$	$L_{-Lost>t25}$	L_{t20}	L_{t40}	$L_{-av>t15}$	$L_{-av>t25}$	L_{v10}	SFV_{t60}	TTF
PC 0.4 µm	ChlA (µg/L)	-0.14	-0.11	-0.33	-0.22	-0.09	-0.07	-0.36	-0.67	0.55
	Cell density (Cells/mL)	-0.04	-0.03	-0.29	-0.21	-0.10	-0.08	-0.44	-0.67	0.63
	Dry weight (mg/L)	-0.03	-0.01	-0.29	-0.21	-0.09	-0.08	-0.44	-0.66	0.60
	TEP (abs/cm/L)	-0.86	-0.85	-0.65	-0.50	-0.48	-0.44	-0.26	-0.68	0.60
	cTEP (abs/cm/L)	-0.90	-0.90	-0.67	-0.58	-0.61	-0.59	-0.46	-0.79	0.87
	tot sTEP	-0.95	-0.94	-0.71	-0.58	-0.60	-0.57	-0.41	-0.80	0.82
	bTEP (abs/cm/L)	-0.66	-0.67	-0.66	-0.69	-0.75	-0.75	-0.71	-0.62	0.73
	bcTEP (abs/cm/L)	-0.66	-0.67	-0.49	-0.53	-0.63	-0.64	-0.27	-0.07	0.08
	tot bTEP	-0.76	-0.78	-0.65	-0.70	-0.79	-0.79	-0.53	-0.36	0.42
	EPSps (mg/L)	-0.64	-0.64	-0.55	-0.44	-0.42	-0.39	-0.49	-0.90	0.97
	SMP_{ps} (mg/L)	-0.87	-0.86	-0.79	-0.69	-0.66	-0.63	-0.46	-0.69	0.55
	bTEP/TEP	0.37	0.35	0.50	0.37	0.25	0.23	0.44	0.80	-0.68
	Fe	-0.33	-0.32	-0.41	-0.42	-0.39	-0.39	-0.21	0.01	-0.24
	Mg	-0.96	-0.96	-0.99	-0.96	-0.94	-0.93	-0.93	-0.90	0.82
	Ca	-0.91	-0.90	-0.75	-0.61	-0.56	-0.52	-0.46	-0.81	0.67
	Total CH	-0.90	-0.89	-0.81	-0.68	-0.65	-0.61	-0.56	-0.93	0.89
	Total TEP	-0.97	-0.98	-0.76	-0.68	-0.72	-0.70	-0.49	-0.73	0.77
PC 0.1 µm	ChlA (µg/L)	0.69	0.62	-0.53	-0.53	-0.42	-0.44	-0.71	-0.56	0.45
	Cell density (Cells/mL)	0.59	0.58	-0.67	-0.64	-0.50	-0.51	-0.77	-0.69	0.64
	Dry weight (mg/L)	0.63	0.61	-0.65	-0.61	-0.47	-0.48	-0.75	-0.67	0.60
	TEP (abs/cm/L)	-0.22	-0.36	-0.37	-0.49	-0.59	-0.61	-0.51	-0.43	0.34
	cTEP (abs/cm/L)	-0.61	-0.67	-0.71	-0.78	-0.87	-0.86	-0.69	-0.76	0.78
	tot sTEP	-0.49	-0.59	-0.61	-0.71	-0.82	-0.82	-0.66	-0.68	0.65
	bTEP (abs/cm/L)	-0.59	-0.58	-0.80	-0.79	-0.83	-0.81	-0.63	-0.77	0.83
	bcTEP (abs/cm/L)	-0.76	-0.80	-0.05	-0.12	-0.28	-0.27	0.05	-0.02	0.02
	tot bTEP	-0.79	-0.81	-0.44	-0.47	-0.60	-0.59	-0.29	-0.40	0.43
	EPS_{ps} (mg/L)	-0.21	-0.25	-0.86	-0.89	-0.88	-0.88	-0.88	-0.92	0.94
	SMP_{ps} (mg/L)	-0.10	-0.25	-0.42	-0.53	-0.62	-0.63	-0.55	-0.44	0.31
	bTEP/TEP	-0.53	-0.43	0.61	0.65	0.58	0.59	0.80	0.66	-0.54
	Fe	0.83	0.63	0.20	0.14	0.07	0.06	0.10	0.26	-0.42
	Mg	-0.13	-0.55	-0.88	-0.91	-0.93	-0.93	-0.91	-0.84	0.74
	Ca	0.37	-0.19	-0.53	-0.62	-0.67	-0.69	-0.71	-0.57	0.43
	Total CH	-0.18	-0.29	-0.74	-0.82	-0.88	-0.88	-0.83	-0.79	0.71
	Total TEP	-0.63	-0.72	-0.61	-0.70	-0.82	-0.82	-0.60	-0.65	0.64
PES 5 kDa	ChlA (µg/L)	-0.92	-0.92	-0.86	-0.77	-0.79	-0.77	-0.86	-0.84	0.85
	Cell density (Cells/mL)	-0.92	-0.99	-0.89	-0.81	-0.84	-0.82	-0.84	-0.88	0.91
	Dry weight (mg/L)	-0.94	-0.99	-0.89	-0.81	-0.84	-0.82	-0.85	-0.88	0.92
	TEP (abs/cm/L)	0.01	-0.07	-0.33	-0.28	-0.31	-0.30	-0.41	-0.31	0.20
	cTEP (abs/cm/L)	0.17	-0.11	-0.36	-0.32	-0.37	-0.38	-0.35	-0.36	0.26
	tot sTEP	0.11	-0.10	-0.38	-0.32	-0.37	-0.38	-0.40	-0.36	0.25
	bTEP (abs/cm/L)	0.17	-0.13	-0.37	-0.42	-0.44	-0.47	-0.31	-0.38	0.33
	bcTEP (abs/cm/L)	0.75	0.65	0.31	0.20	0.22	0.19	0.25	0.29	-0.39
	tot bTEP	0.57	0.35	0.01	-0.08	-0.08	-0.11	0.00	0.00	-0.09
	EPS_{ps} (mg/L)	-0.31	-0.57	-0.68	-0.58	-0.65	-0.65	-0.62	-0.66	0.62
	SMP_{ps} (mg/L)	-0.07	-0.12	-0.43	-0.43	-0.43	-0.43	-0.53	-0.42	0.31
	bTEP/TEP	0.81	0.85	0.88	0.79	0.82	0.80	0.90	0.86	-0.84
	Fe	0.05	0.30	-0.03	-0.14	-0.07	-0.07	-0.20	-0.03	-0.04
	Mg	-0.81	-0.78	-0.95	-0.99	-0.97	-0.97	-0.98	-0.96	0.94
	Ca	-0.85	-0.63	-0.75	-0.68	-0.70	-0.68	-0.84	-0.73	0.67
	Total CH	-0.21	-0.39	-0.65	-0.59	-0.63	-0.63	-0.68	-0.63	0.53
	Total TEP	0.27	0.04	-0.29	-0.28	-0.31	-0.33	-0.31	-0.28	0.17

Appendix C

Appendix of Chapter 5: Decreasing membrane fouling during *Chlorella vulgaris* broth filtration via membrane development and coagulant assisted filtration

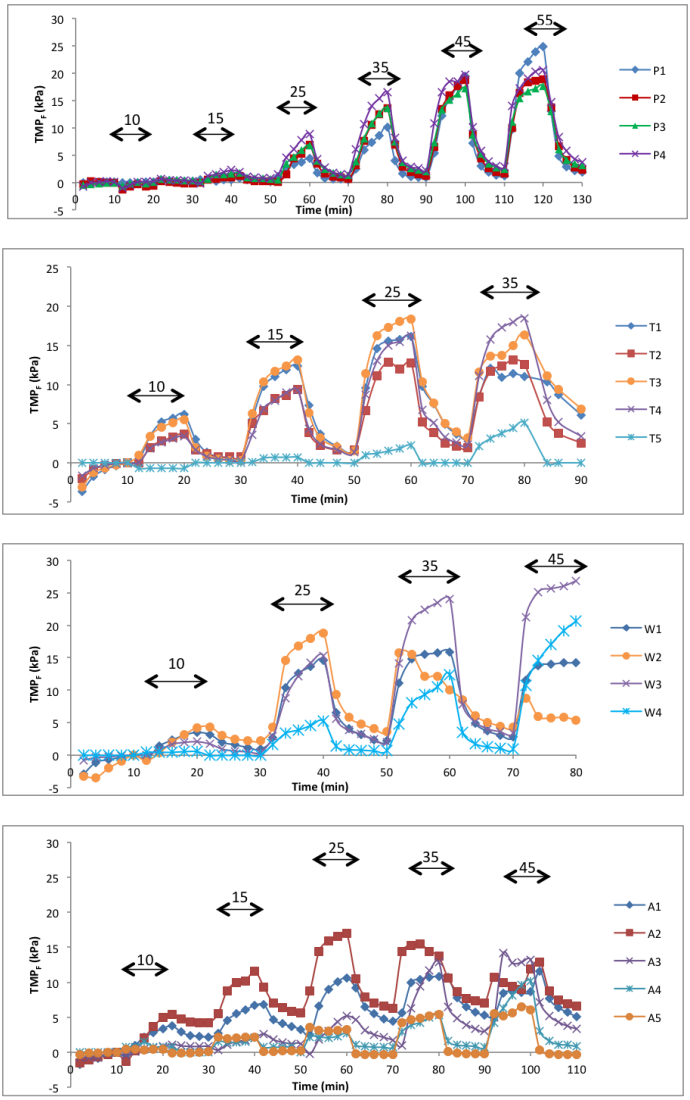

Figure C.1: Flux stepping profile of membranes from top to bottom: P-, T-, W- and A-series.

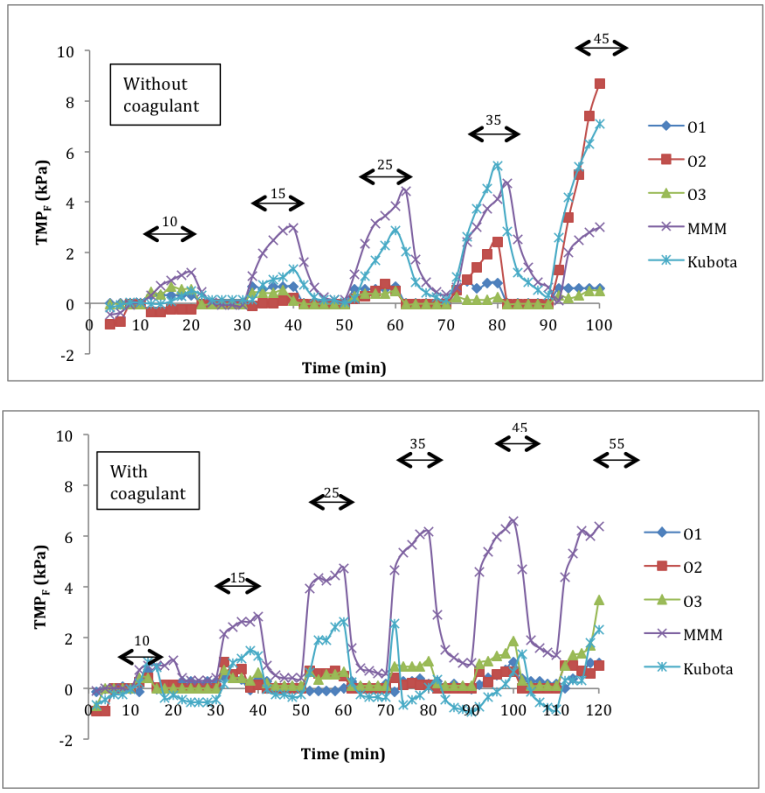

Figure C.2: Flux stepping profiles of the selected membranes (O1-O3) and two commercial membranes (MMM from Amer-Sil and PE from Kubota) (lower graph) without (upper graph) with chitosan (10 ppm) as coagulant.

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Marbelia, L., Bilad, M.R., Passaris, I., Discart, V., Vandamme, D., Beuckels, A., Muylaert, K., and Vankelecom, I.F.R. Membrane photobioreactors for integrated microalgae cultivation and nutrient remediation of membrane bioreactors effluent. Submitted – Bioresource Technology.

Meeting abstracts, presented at national and international scientific conferences and symposia, published or not published in proceedings or journals

International conference and exhibition "Desalination for the environment: clean water and energy", April 22-26 2012, Barcelona, Spain. Oral presentation: The influence of transparent exopolymer particles on membrane fouling in water purification and algae filtration

NMG-BMG Poster day, June 7, 2012, Amsterdam, Netherlands. Poster

Methusalem Advisory Board Meeting Heverlee, Belgium, October 26, 2012, poster presentation: V. Discart, D. Vandamme, I. Foubert, K. Muylaert, I. Vankelecom; Simultaneous cultivation and pre-harvesting of microalgae in a labscale membrane photobioreactor

Workshop on membranes, catalysts and nanomaterials for advanced water treatment, April 16th 2014, Teri University, New Delhi, India, Oral presentation: I. Vankelecom, V. Discart, L. Marbelia, R. Bilad; Algal-MBR for wastewater treatment

FACULTY OF BIOSCIENCE ENGINEERING
DEPARTMENT OF MICROBIAL AND MOLECULAR SYSTEMS
CENTER FOR SURFACE CHEMISTRY AND CATALYSIS
Kasteelpark Arenberg 20
3001 LEUVEN, BELGIUM
valerie.discart@biw.kuleuven.be
www.biw.kuleuven.be